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Original Article

Androctonus australis hector Venom Induced Immuno-Allergic Type Response: Comparison with an Experimental Model of Allergy

Le Venin d'Androctonus australis hector Induit une Réponse de Type Immuno-Allergique : Comparaison avec un Modèle Expérimental d'Allergie

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ABSTRACT

Introduction: The physiopathology of scorpion envenoming is very complex, and can induce several perturbations reaching different systems, including nervous, cardiovascular, immune and respiratory systems. Envenoming by *Androctonus australis hector (Aah)* scorpion is characterized by various symptoms, including pain and cardiovascular and respiratory disturbances. In the most severe cases of envenomation, pulmonary edema could be responsible of death. **Materials and Methods:** This study was carried out as a comparative analysis of the immuno-inflammatory response of envenomed mice with that of an experimental model of allergy. In a purpose of assessing the damage caused by *Aah* venom or its components on the respiratory system, we update an allergic model in mice using a pretreatment with ovalbumin (OVA) for a period of 15 days intra-peritoneally and then intra-nasally. On the other hand, three groups of mice were envenomed by the whole *Aah* venom, its toxic fraction (FtoxG-50), or its non toxic fraction (F1) subcutaneously. **Results:** The inflammatory response was assessed by evaluating the pulmonary vascular permeability, measuring the levels of IgE in sera, and histo-pathological study of lung parenchyma. Our results showed an enhancement in pulmonary vascular permeability, with important changes in the lung parenchyma, including wall thinking, accompanied with IgE synthesis, observed in mice pretreated with ovalbumin. The same results were obtained with envenomed groups of mice. **Conclusion:** The inflammatory pattern of the experimental model of allergy was comparable to those of envenomed groups. The scorpion venom may play an important role in mediating inflammatory response allergic type, in activating immune and non-immune cells and mediators that trigger the allergic disorder. Thus, it should be interesting to investigate the properties of venom components, to learn about the mechanisms by which they stimulate effector cells and inflammatory mediators, which could be used in a therapeutic side.

KEYWORDS: Allergy, Experimental model of mice, Ovalbumin, Lung, Scorpion venom.

RESUME

Introduction: La physiopathologie de l'envenimation des scorpions est très complexe et peut induire plusieurs perturbations atteignant différents systèmes, notamment les systèmes nerveux, cardiovasculaire,

immunitaire et respiratoire. L'envenimation du scorpion d'*Androctonus australis hector (Aah)* se caractérise par divers symptômes, notamment des douleurs et des troubles cardiovasculaires et respiratoires. Dans les cas d'envenimation les plus sévères, l'œdème pulmonaire peut être la cause du décès. **Matériels et Méthodes :** Cette étude a été réalisée comme analyse comparative de la réponse immuno-inflammatoire de souris envenimées avec celle d'un modèle expérimental d'allergie. Dans le but d'évaluer les dommages causés par le venin d'*Aah* ou ses composants sur le système respiratoire, nous avons optimisé un modèle allergique chez la souris en utilisant un prétraitement à l'ovalbumine (OVA) pendant une période de 15 jours par voie intra-péritonéale puis intra-nasale. D'autre part, trois groupes de souris ont été envenimés par le venin total d'*Aah*, sa fraction toxique (FtoxG-50) ou sa fraction non-toxique (F1) par voie sous-cutanée. **Résultats :** La réponse inflammatoire a été évaluée par la mesure de la perméabilité vasculaire pulmonaire, la quantification des niveaux sériques d'IgE, et l'étude histo-pathologique sur parenchyme pulmonaire. Nos résultats montrent une augmentation de la perméabilité vasculaire pulmonaire, avec des changements importants dans la structure du parenchyme pulmonaire, avec un épaississement des parois alvéolaires, accompagnés d'une synthèse d'IgE, observés chez des souris prétraitées avec de l'ovalbumine. Les mêmes résultats ont été obtenus avec des groupes de souris envenimées. **Conclusion :** Le profil inflammatoire du modèle expérimental d'allergie était comparable à ceux des groupes envenimés. Le venin de scorpion peut jouer un rôle important dans la médiation d'une réponse inflammatoire de type allergique, dans l'activation des cellules immunitaires et non immunitaires et des médiateurs qui déclenchent le trouble allergique. Ainsi, il serait intéressant d'étudier les propriétés des composants du venin, afin de mieux comprendre les mécanismes par lesquels il stimule les cellules effectrices et les médiateurs inflammatoires qui pourraient être utilisés dans le domaine thérapeutique.

MOTS CLES : Allergie, Modèle expérimental de souris, Ovalbumine, Poumon, Venin de scorpion.

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1. Introduction

In the lungs, edema is one of the most frequent complications in scorpionic accidents, and the principal cause of death in the majority of cases. The pulmonary edema can be induced by two types of factors: a cardiogenic factor directly related to a cardiac dysfunction of the left ventricle, and a non-cardiogenic factor caused by the release of inflammatory mediators [1].

Cardiogenic origin of edema could be due to an ischemia state related to catecholamine and acetylcholine discharge, or biventricular failure leading to left ventricular dysfunction [1,2]. The non-cardiogenic or lesional edema could be due to an increase in membrane permeability resulting from the alveolar capillary membrane lesion and activation of inflammatory cascade by mediators, such as prostaglandins, leukotriens, histamine and cytokines [3].

The bioactive substances of scorpion venom are neurotoxic peptides, present in small amounts, but responsible for almost all the toxicity of *Aah* venom, due to their capacity to act on the voltage-gated sodium channels of excitable cells; thus, they can induce various disturbances, such as the depolarization of the axonal membranes, and a consequent release of neuromediators, which have deleterious effects on different organs [4-6]. In the lung, the physio-pathological effects of the envenomation seem to be related to the alteration of the alveolo-capillary barrier, resulting a pulmonary vascular permeability increase and remarkable leukocyte infiltration, accompanied by the activation of the inflammatory cascade, with significant lesions of the pulmonary parenchyma [7,8]. These manifestations occurring in the lung following the envenomation suggests other disorders affecting this organ, which could be similar to an allergic type disorder.

Allergy is a hypersensitivity reaction, initiated by adaptive immunological mechanisms. It can be antibody- or cell-mediated. In the majority of cases, the antibody typically responsible for an allergic reaction belongs to the IgE isotype, and these individuals may be referred to as suffering from an IgE-mediated allergy [9,10]. The present study was designed to better understand the mechanisms involved in the immuno-reactivity of the respiratory tract, a comparison was then established between envenomed groups of mice with the whole venom of *Aah*, or one of its components, and an experimental model of allergy induced with ovalbumin.

2. Material and Methods

1. Scorpion venom and its fractions

F1 and FtoxG-50 fractions were isolated from *Aah* venom by gel filtration through Sephadex G50 column as previously described, and were provided from the Laboratory of Cellular and Molecular Biology, Faculty of Biological Sciences of USTHB [11].

2. Animals

Adult male NMRI mice with an average weight of 20 ± 2 g were purchased from the animal breeding of the Pasteur Institute of Algeria. They were housed in temperature and humidity controlled rooms and received food and water *ad libitum* before being used for study. The experiments were achieved in line with the current guidelines for the care of laboratory animals [12].

3. Experimental protocol

In a purpose of assessing the damages caused by *Aah* venom, or its components on the respiratory system, we developed an allergic murine model using a pretreatment with ovalbumin (OVA) for a period of 15 days [13-15]. First, mice received intra-peritoneally (i.p. route) doses of 10 µg each of OVA, in a solution containing 0.133 mg of $Al(OH)_3$ on the first, second and third day of pretreatment. The OVA was then administered intra-nasally, without adjuvant, at doses of 2 µg each on the sixth, seventh, fourteenth and fifteenth day of the pretreatment. In a second set of experiment, three groups of mice were envenomed by a sublethal dose of the whole *Aah* venom (10 µg/20 mg of body weight), its toxic fraction (FtoxG-50) (8 µg/20 mg of body weight), or its non-toxic fraction (F1) (8 µg/20 mg of body weight) subcutaneously, and are considered as positive controls of envenomation [16,17]. The negative control is represented by a group of mice injected with physiological saline solution (0.9% NaCl).

4. Determination of pulmonary vascular permeability

In all groups, the lungs were removed at the end of each experiment to determine the pulmonary water content by a method already described and modified [18]. Briefly, the lungs were excised, weighed and dried to a constant weight in an oven at a temperature of 37°C. The difference between wet and dry weight of the lung provided the water content, and was expressed as a percentage of wet lung tissue.

5. Evaluation of IgE concentration in the serum

The IgE level in serum sample was determined by an enzyme-linked immune-sorbent assay method [19]. A 96-well microplate was sensitized with a monoclonal antibody anti-mouse IgE at a concentration of 5 µg/mL dissolved in a 0.1 M carbonate buffer, pH 9.6 at a rate of 100 µL/well, and then incubated overnight at 4°C. After washing with PBS, the non-specific sites are saturated with 3% skimmed milk prepared in PBS for 3 h at 37°C. The serum samples are deposited in triplicate at the rate of 100 µL per well, and the microplate was incubated overnight at 4°C. A solution containing 2 µg/mL of biotinylated anti-IgE antibodies was then deposited. After an incubation of 90 min at 25°C, the streptavidin-peroxidase was added. The peroxidase substrate, composed of a 0.01 M phosphate buffer, pH 7.4 containing 10 µL of H_2O_2 and 1 mg/mL of O-phenylenediamine (OPD), was added to each well, and the microplate was incubated at room temperature for 30 min. After stopping the reaction by adding 50 µL of 30% H_2SO_4.The absorbance was read at 490 nm. The result is expressed in ng/mL.

6. Characterization of histo-pathological effects

The analysis of tissue integrity was performed on the lungs of control, envenomed and pretreated animals. The lungs were carefully removed after the sacrifice of mice, and immediately immersed in formalin at 4% for 48h, then dehydrated in ethyl alcohol, and impregnated in paraffin before making blocks. Sections of 4 µm thick were made using a rotary microtome, and then spread on glass slides. The sections were subjected to topographic staining with Hematoxylin-Eosin, and observed under light microscopy at ×400 magnification.

7. Statistical analyses

The data were reported as mean ± standard error of the mean (S.E.M.). The statistical significance of differences between groups was analyzed by a Student *t* test. Differences were considered significant if probability values (P) were 0.05 or less.

3. Results

1. Determination of pulmonary vascular permeability

The extent of pulmonary edema was estimated by determining the water content of the lungs in mice envenomed with *Aah* venom, its toxic fraction (FtoxG-50), or its non-toxic fraction (F1). These levels was compared to this obtained with the experimental modal of allergy. The results are expressed as a percentage of wet weight **(Figure 1)**.

The results obtained show an increase in the pulmonary water content in the mice envenomed with the whole venom or one of its fractions, by comparing with those of the negative control. Indeed, fairly large values were recorded for the lungs of mice after envenomation with the fraction F1 (85.859 ± 8.54 %), the closer result to those obtained with OVA-treated mice (90.5 ± 4.7 %). The increase in these reports reflects the formation of exudate in the lungs and confirms the formation of pulmonary edema **(Figure 1)**.

2. Evaluation of IgE concentration in the serum

The IgE level was measured in the sera of mice after subcutaneous injection of a sublethal dose of the whole venom, its toxic fraction (FtoxG-50) or its non-toxic fraction (F1), and then compared to the level obtained with OVA-treated mice **(Figure 2)**. The inflammatory profile of the allergic model was marked by a high production of IgE in the serum compartment, with a concentration of 34.1±3.11ng/mL. When compared to envenomed groups of mice, the higher concentration of IgE was registered in mice injected with F1 fraction (31.2±6.54ng/mL), which is greater than that induced by *Aah* venom (28.4±3.78ng/mL) or FtoxG-50 fraction (26.8±1.26 ng/mL) **(Figure 2)**.

3. Characterization of histo-pathological effects

Inflammatory response was also assessed by evaluating the pulmonary edema and the alterations of lung parenchyma **(Figure 3)**. Anatomo-pathological analysis of the lungs of mice having received a sublethal dose of the venom or one of its fractions, highlighted a damage affecting the alveolar and inter-alveolar space, representing by a complete disorganization of the structure of the pulmonary parenchyma, with edema, hemorrhagic areas, infiltration of inflammatory cells as well as thickening of the intra-alveolar walls comparing with control **(Figure 3a)**. This tissue damage was most important for the injected animals with toxic fraction **Figure 3d)**. Our data showed a similar profile comparing the allergic model of mice **(Figure 3b)** with envenomed groups of animals **(Figure 3c, 3d and 3e)**.

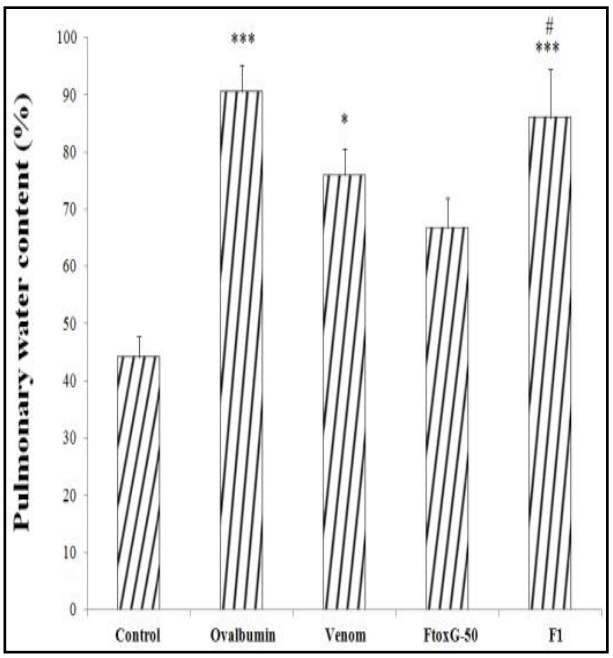

Figure 1: Estimation of pulmonary edema by calculating the wet weight of the lungs of mice 4 h after experimental envenomation with the whole venom of *Aah* (0.5 mg/kg), the FtoxG-50 fraction (0.4 mg/kg) or the F1 fraction (0.4 mg/kg), by comparing with an allergic model (mean ± standard deviation, n = 4).

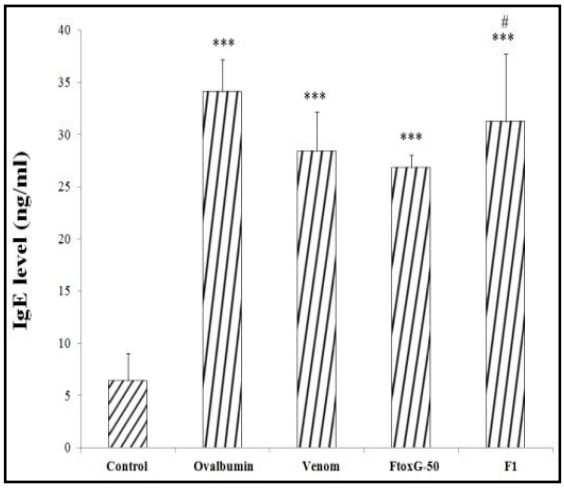

Figure 2: Evaluation of serum IgE concentrations, 4 h after experimental envenomation with the whole venom of *Aah* (0.5 mg/kg), the FtoxG-50 fraction (0.4 mg/kg) or the F1 fraction (0.4 mg/kg), by comparing with an allergic model (mean ± standard deviation, n = 4).

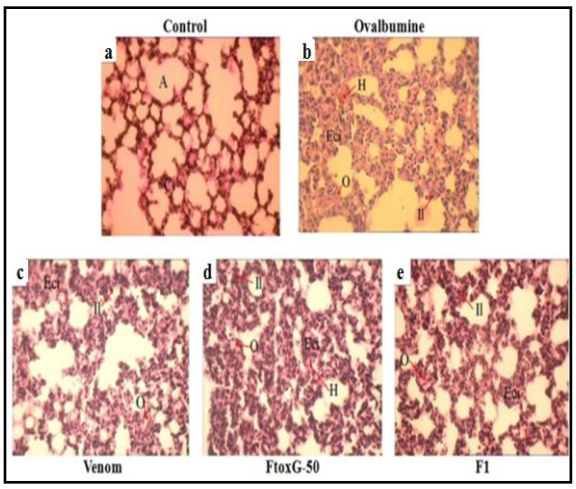

Figure 3: Light photomicrographs of the lungs of mice treated with ovalbumin (b), or injected with *Aah* venom (c), FtoxG-50 fraction (d) or F1 fraction (e) showed an increase in cellular infiltration and an obstruction of airways comparing with control (a).

Alveolus **(A)**, hemorrhage **(H)**, edema **(O)**, leukocyte infiltration **(II)**.Thickening of the inter-alveolar partitions **(Eci)**.Hematoxylin-eosin stained. ×400 magnification.

4. Discussion

The increase in vascular permeability is an early eventobserved in venom-induced inflammation [20,21]. It is accompanied by heartfailure, cardio-respiratory disorders, inflammatory cells infiltration with high levels of cellular enzymatic activity and pro-inflammatory cytokines (IL-1, IL-6 and TNFα) [17,22,23].

Pulmonary edema is associated with acute left ventricular dysfunctionand damage, caused by cardiogenic components. It could also result from an increase incapillary lung permeability by non-cardiogenic components [24,25]. The latter appears to be related to an increase of pulmonary vascular permeability that accompanies activation of the inflammatory cascade [25].

The mast cell has been known to be an important cell type involved in IgE-mediated immediate hypersensitivity and allergic disorders [26-28]. Mast cells are activated through their IgE-bound high affinity IgE receptors (FcεRI), and release the preformed pro-inflammatory mediators [9]. They are also activated by the tachykinin substance P (SP), to induce various effects, such as regulation of neurotransmission, pain, inflammation, cell growth and differentiation and oncogenesis [29].

It has been demonstrated that mast cells are involved in the lung edema induced by scorpion venoms of different species [30,31]. They are also involved in the inflammatory response caused by other types of venoms, such as snake, bee, spider or wasp venoms [21,32-36]. The contribution of neuropeptides, in particular substance P, and the role of tachykinin (NK1) receptor in the lung injury induced by *Tityus serrulatus* scorpion venom has been also demonstrated [37].

We have previously evaluated the increase in microvascular permeability, the edema formation, the cellular recruitment, the structural lung disorganization and the role of tachykinin molecules in mast cell activation following the administration of *Aah* venom or its components [38].

The allergic reaction is an immune system's response to the inoculation of an allergen. This immune response is initiated during exposure to the allergen, then its recognition by the immune system, and completed by the production and interaction of IgE with effector cells, mainly mast cells, which released mediators that activate other inflammatory cells, responsible for various manifestations, including vasodilation, hemodynamic variations, broncho-constriction and increased mucus secretion [39-41].

The particularity of hypersensitivity response compared to normal immune response is the IgE production. This isotype of antibody binds to the FcεRI receptors on the surface of resident mast cells and blood basophils, which initiates a complex intracellular signaling cascade that leads to degranulation and the release of pharmacologically active mediators, such as histamine, lipid metabolites and cytokines. The principal effects of these products are vasodilatation, increased vascular permeability, and smooth muscle contraction, which may act locally or systemically [9,42,43].

We have previously demonstrated that the phenomenon of plasma extravasation after the administration of *Aah* venom, its toxic fraction or non-toxic fraction, promotes the installation of pulmonary edema, following the alteration of the functions of the lung, and the increase in vascular permeability to serum proteins. These damages are accompanied by a massive leukocyte infiltration in the broncho-pulmonary space, and the alteration of the lung parenchyma). We have also underlined the important role of mast cells in mediating the inflammatory response through their NK1 receptors [38]. In this study, the pulmonary immune-reactivity

induced by the constituents of *Aah* venom was compared to that of an experimental model of allergy developed in the laboratory.

The comparative analysis of the immuno-inflammatory response of envenomed mice with an experimental model of allergy showed that the edema induced by *Aah* venom or its components is almost similar to that observed with the experimental model of allergy. *Aah* venom seems to trigger an inflammatory reaction allergic type with high levels of IgE production.

Broncho-pulmonary hyper-responsiveness is a complex phenomenon involving multiple cell populations, including neutrophils, lymphocytes, macrophages, eosinophils, possibly stromal cells and bronchial epithelium. The cell interactions allow mast cells to orchestrate inflammatory innate reactions and complex adaptive immunity, including the pathogenesis of allergies.

One of the parenchymal tissues most sensitive to the action of scorpion venoms is the lung. Its structure is characterized by a large alveolar surface, a high blood flow and an ease in the exchanges between the alveolar air and the vascular compartment, allowing a rapid absorption and excretion of toxic agents. These characteristics could therefore explain the severity of the lung damage observed after scorpion envenoming [44]. The sequestration of polynuclear cells in the lungs and the release of free radicals, reported in several studies, seem to be responsible for lesions of the pulmonary parenchyma of envenomed animals [7,45].

Our results highlight the role of *Aah* venom components in inducing changes in the structure of the lung parenchyma, added to the variation in vascular permeability, and thus the accumulation of inflammatory cells and their products in the broncho-pulmonary space. The production of high levels of IgE suggests that the mast cell signaling could be involved through their FcεRI receptor. This inflammatory pattern is almost similar to the manifestations observed with allergic model, which suggests that *Aah* venom components could induce an inflammatory response allergic type.

The comparative analysis of the immuno-inflammatory response of envenomed mice with an experimental model of allergy constitutes a fundamental tool for a better understanding of the pathophysiology of venomous pathogens. However, the effects of the immuno-allergic response affecting the broncho-pulmonary space as well as the production of IgE, seem to be accentuated under the action of the non toxic fraction (F1). The presence of allergenic biomolecules in this fraction is to be explored.

Conclusion

In summary, the obtained results indicate that *Aah* venom may play an important role in mediating inflammatory response allergic type, in activating cell types, such us mast cells, and mediators that trigger the allergic disorder. Thus, it should be interesting to investigate the properties of venom components, to elucidate the mechanisms by which they stimulate effector cells and inflammatory mediators, which could be used in therapeutic purposes.

Conflicts of interest

Authors do not declare any conflict of interest.

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