Expression and immunological characterization a heat shock cognate-70 protein allergen, rAed a 8, from the mosquito species *Aedes aegypti*

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**Background:** *Aedes aegypti* is the source of saliva as well as somatic allergens. An IgE-binding Heat shock cognate-70 protein (Aed a 8) from this mosquito, has been described as a somatic allergen. Its biological and immunological properties have been scarcely studied.

**Objective:** To perform the immunological characterization of the recombinant allergen, rAed a 8.

**Methods:** Recombinant Aed a 8 was expressed in *Escherichia coli*, purified and its IgE-binding capacity was evaluated by ELISA and immunoblotting using the sera from 14 individuals residing on the tropical island of Martinique, with positive skin test against *Aedes communis* whole body extract. The allergenicity of rAed a 8 was studied by the Basophil Activation Test and ELISA competition. For the Basophil Activation Test, stripped basophils re-sensitized with IgE from a rAed a 8 sensitized patient were used. These cells were incubated with rAed a 8 and CD63 up-regulation was assessed by flow cytometry. BALB/c mice were immunized with rAed a 8, or PBS, and specific IgE, IgG1, IgG2a and IgG3 were measured. For detection of IgG1, IgG2a and IgG3 sera were diluted 1:500; and for IgE 1:12. Murine IgG1 binding of the whole extract was verified by ELISA. T-cell activation was studied in splenocytes stimulated with rAed a 8.

**Results:** rAed a 8 was expressed as a 74 kDa protein linked to a His-tag. The recombinant was purified by affinity, reacted with specific-IgE in 6 out of 14 (43 %) allergic individuals and induced the activation of basophils, suggesting that is an important allergen that could be considered in diagnostic tests and in specific-immunotherapy for mosquito allergy. When the *Aedes aegypti* extract was on the solid phase, rAed a 8 produced 40 % of inhibition of the IgE reactivity at the highest concentration. When rAed a 8 was on the solid phase, the extract produced a similar degree of inhibition. rAed a 8 induced the production of specific IgE, IgG1, IgG2a and IgG3
antibodies. At the same dilution of the mice sera, the levels of IgG2a were higher than the levels of the other IgG subclasses. Murine IgG1 showed reactivity with the mosquito extract in ELISA. rAed a 8 induced proliferation of mice splenocytes.

Conclusions: rAed a 8 was expressed as a biological active protein. The allergen induced a mixed antibody response characterized by the production of Th1 and Th2 related antibodies. Further studies in well-defined allergic populations are needed to define whether Aed a 8 is a major or minor allergen.

Keywords: Aedes aegypti; Recombinant allergen