



# GENERATION AND CHARACTERIZATION OF A PANEL OF SPECIFIC MONOCLONAL ANTIBODIES AGAINST CANINE IgE USING RECOMBINANT IMMUNOGENS

**AUTHORS:** García-Gallo Pinto, M. 1; Llorente Gómez, M. 1; Martín López, L. 1; Kremer Barón, L. 1, Mas Fontao, A. 2, Álvarez Álvarez, J. 2

**WORK CENTERS:** [1] *Protein Tools Unit & Department of Immunology and Oncology, CNB-CSIC, Cantoblanco* – Madrid Tlf. 915854570; E-MAIL: lkremer@cnb.csic.es; [2] *ALERGOVET S.L.; C/ Luis Cabrera 92- 28002* – Madrid. Tlf. 914134472; E-MAIL: jalvarez@alergovet.com

**OBJECTIVE:** The design of an effective treatment of allergy in dogs needs the individual determination of those allergens related with the disease. This can be made through the detection of specific immunoglobulin E (IgE) in serum, using *in vitro* techniques. One of the most important problems in the study of the canine immune system is the lack of antibodies to study its components. So, few works develop specific, sensitivity and effective reagents for the detection of canine IgE, which represents 0.003% approximately<sup>[1]</sup> of the circulating immunoglobulins in dogs. Due to the great difficulty to obtain preparations of IgE completely free of IgG, major immunoglobulin, many of the reagents available show cross-reactivity with IgG, producing false positives results and, therefore, a mistaken diagnosis. In order to solve this problem, the aim of this work was the development of new monoclonal antibodies (mAb) against canine IgE, using in its generation a recombinant IgE antigen absolutely free of IgG, and an exhaustive molecular characterization of those selected anti-canine IgE mAbs.

**MATERIALS AND METHODS:** A molecule of recombinant canine IgE (rIgE) produced in Alergovet, which comprises the domains CH2-CH3-CH4 of the constant region of the heavy chain of canine IgE<sup>[2]</sup>, was used as immunogen for the generation of mAbs. rIgE was conjugated with keyhole limpet hemocyanin, emulsified with adjuvant of Freund, and injected subcutaneously to a mice of the strain BALB/c. The selection of immunized mice was made by titration of antibodies anti-rIgE in mice's sera using direct ELISA against rIgE. In order to generate hybridomas, spleen lymphocytes were extracted from the mouse selected as better responder, and merged with murine myeloma cells. The presence of specific antibodies was studied by ELISA against rIgE test and bovine albumin as negative control. Hybridomas that presented the best relation signal versus background were cloned by two successive rounds of limiting dilution. The selection of hybridomas was made assaying cellular supernatants with two methods: direct ELISA using rIgE as antigen and indirect ELISA to assay the amount of specific IgE in sera of allergic dogs and serum of healthy dogs (negative control). Cross-reactivity of mAbs with canine-IgG and IgM was studied. Those mAb without recognition of canine-IgG/IgM were selected. Finally, ELISA competitive assay using non-conjugated mAbs as competitor was tested to study mAb epitope reactivity.

**RESULTS:** The study of sera of immunized mice demonstrated a good level of antibodies against rIgE, being used for cellular fusion purposes the mouse with highest title of antibodies. The supernatants of more than 3,000 hybridomas were analyzed and the positives in this test were studied with an indirect ELISA to assay specific IgE in sera of dogs, using different allergens as antigen. Twenty hybridomas contained mAb specific for rIgE with capacity to recognize native IgE. Hybridomas of four of these wells were cloning through two rounds of limiting dilution. All mAbs showed high canine-IgE specificity without cross-reactivity with dog purified IgG/IgM. ELISA competitive assay showed that the target epitope for each mAb did not overlap.

**CONCLUSIONS:** Alergovet in collaboration with CNB-CSIC has developed a panel of monoclonal antibodies specific for canine-IgE using a recombinant antigen. These mAbs did not recognize canine IgG/IgM, avoiding false positives. In addition, all mAbs are able to detect specific IgE in sera from allergic dogs when major allergens related with canine allergy were tested. Finally, it has been shown that mAbs interact with different epitopes of IgE molecule, allowing its use in a oligoclonal mixture, a novel molecular tool for the determination of specific IgE in dogs, which increases the sensitivity of the technique maintaining its' specificity.

## REFERENCES:

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This work is funded by:

