# ImmunoTools *special* Award 2022

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## CD34$^{+}$ - DERIVED MAST CELLS CHARACTERIZATION FROM HEALTHY VOLUNTEERS AND ALLERGIC PATIENTS

Our group is interested in the development of a mast cell activation system from blood progenitors differentiated in culture from healthy and allergic patients to improve the current diagnostic techniques. Also, we want to study the differences between mast cells from these patients and mast cells from healthy controls in terms of degranulation and mediator production. And finally, we are interested in the new population of Thf13 in allergic patients.

In this study, we propose the analysis of the signaling pathways and their effects on mast cell activation in spontaneous in healthy and allergic individuals. For this purpose, the following will be obtained from control and allergic patients and differentiated in culture to mast cells (*Alvarez-Errico, D et al. J. Immunology 2011; Ainsua-Enrich, E at al. J. Immunology 2012; Ainsua-Enrich, E at al. J. Immunology 2015*). Subsequently, the mast cell activation test (MAT) will be developed and validated. This test consists in obtaining differentiated mast cells *in vitro* from peripheral blood precursors, their subsequent passive sensitization with sera from allergic patients and *in vitro* incubation with the allergen under study leading to the release of inflammatory mediators concomitantly with the expression of CD63 (granule marker) on the plasma membrane of the mast cell. The determination of CD63 is a marker of mast cell activation. This test has demonstrated a diagnostic accuracy in distinguishing different phenotypes of food allergy than basophil tests carried out in the basophil test commonly performed in the clinic (*Bahri R. et al., J Allergy Clin Immunol. 2018; Santos AF et al. J Allergy Clin Immunol, 2018*). The use of this test will allow us to *in vitro* evaluation of allergen reactivity, as well as the study of the signaling pathways involved. The final interest, apart from deepening the knowledge of the mechanisms involved in anaphylaxis, can also be directly clinical, allowing us to obtain a test that will allow us to differentiate the clinical phenotypes with certainty and thus to avoid the exposure tests that entail a not negligible risk in these patients.

To validate the *in vitro* mast cell activation test (MAT), we follow the method used by *Bahri R. et al. (J Allergy Clin Immunol. 2018*). From peripheral blood of healthy volunteers, $CD117^+$ and $CD34^+$ cells (progenitor cells) will be purified from mononuclear cells of the leukocyte layer of the blood using a positive selection kit. Cells will be cultured for 4 weeks in Stem-Pro medium supplemented with 100 U/ml of penicillin, 100 mg/ml streptomycin, human IL-6 (50 ng/ml), human IL-3 (10 ng/ml), human stem cell factor (100 ng/ml), and 10 mg/ml human low-density lipoprotein. After 8 weeks, the maturity of the cells is analyzed for quantification of CD117, FcεRI, and CD34 cells, using flow cytometry (*Santos AF et al. J Allergy Clin Immunol, 2018*). Once the mast cells have been obtained and characterized, they will be incubated during 5 days with IL-4 to upregulate FcεRI (*Toru H. et al., International Immunology, 1996*) and then, sensitized with sera from allergic patients and allergen (for the positive control we use anti-human IgE). Mast cell activation will be determined by the expression of $CD63^+$. Subsequently, the test will be optimized for patients with food or hymenoptera allergies. In this case, progenitor-derived mast cells will be obtained from blood of allergic patients.

Finally, to see differences between healthy volunteers and allergic patients, our group is interested to study Thf13 cells. Recently, *Gowthaman U et al. (Science, 2019*) described a new population of Thf13 cells that seems that are responsible of those severe reactions of anaphylaxis in some allergic patients. This population is characterized by the expression of CD3, CD4, CD45RA and CXCR5 in the cell membrane. For this purpose, Thf13 will be obtained from patient's blood and checked by flow cytometry with these antibodies.