

The method for selecting basophils might be determinant in the basophil activation test in patients with mastocytosis

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Mastocytosis is a heterogeneous disorder characterized by the proliferation and accumulation of mast cells (MC) in the skin, bone marrow, and other tissues[1]. Due to massive MC activation and mediators release, subjects with mastocytosis can suffer systemic symptoms including hypotension and shock, flushing, headache, abdominal pain or diarrhea. Serum baseline tryptase correlates with the total MC number and burden, and is increased in many patients. In mastocytosis, MC can be activated by various stimuli including Hymenoptera venom through an IgE-mediated mechanism[2]. There are several reports on patients with SM with a history of severe sting reactions, but with negative venom-specific IgE and skin tests [3]. In such cases other diagnostic methods, such as the basophil activation test (BAT) can be useful[4]. The reports about the usefulness of BAT in patients with negative specific IgE (sIgE) show different results. Some studies find that BAT doesn't provide useful information in this kind of patients [5,6] while other papers describe the opposite[7,8]. Interestingly, different methods for basophils identification were used in these works, and negative results were obtained in those using CCR3 (chemokine CC receptor type 3)[5,6] while positive results were shown using IL3 receptor (CD123) in conjunction with HLA-DR [7] or CD45 and IgE [8].

The usefulness of BAT in two patients with systemic reactions after wasp sting and mastocytosis is described in this work.

Two male patients of 53 and 64 years (patient 1 and patient 2 respectively) with systemic reactions after wasp sting were studied. Patient 1 presented dizziness, vomiting, dyspnoea and loss of consciousness 25 min after the sting, and patient 2 suffered flushing and loss of consciousness 10 min after the sting. None of them suffered cutaneous symptoms. Both showed high tryptase values ($> 20 \mu\text{g}/\text{mL}$) (Table) one month after the reactions.

Both patients showed a REMA score > 2 [9] suggestive of clonal mast cell disorder and were diagnosed of indolent systemic mastocytosis after bone marrow biopsy in the Instituto de Estudios de Mastocitosis de Castilla La Mancha (CLMast) according to World Health Organization (WHO) criteria.

sIgE against whole venoms and components was determined several months after the reaction. Intradermal tests (IDT) with *Apis mellifera*, *Vespula spp.* and *Polistes dominula* venoms up to 1

mcg/ml were also performed in patient 1. The results of sIgE and IDT were negative (Table)

In order to find venom sensitization BAT was performed as previously described [10]. Basophils were detected initially by an anti-IgE monoclonal antibody, and the degranulated basophils by the expression of CD63 on the surface of the cells. The test was carried out against venom from *Polistes dominula* and *Vespula* spp at a final concentration of 1 µg/ mL (Pharmalgen ALK-Abelló, Madrid). BAT results were positive (>15% of activated basophils) in both patients for both venoms (Table) allowing the identification of sensitization and the prescription of venom immunotherapy.

In patient 1 we could repeat BAT six months later using two different membrane molecules (IgE and CCR3) for the identification of basophils. These tests were performed after 6 months of immunotherapy with *Vespula* and *Polistes* venoms. As shown in the table, positive results for *Vespula* and *Polistes* were obtained using IgE, while a low value for *Vespula* and a negative result for *Polistes* were shown using the CCR3 molecule.

In our study, two of two patients diagnosed of SM, with anaphylactic reaction after wasp sting and negative results in the classic diagnostic tests of Hymenoptera venom allergy (HVA), had a positive result in BAT against hymenoptera venom. In the absence of the classic diagnostic tests, this cellular test showed that the reaction was mediated by IgE and allowed the prescription of venom immunotherapy. Venom-specific IgE cannot be detected in 5 -10 percent of patients with mastocytosis and HVA [3]. This fact has been attributed to increased absorption of circulating IgE by the large amount of mast cells, with consequent low circulating levels of sIgE that prevent their proper detection with the classic tests. This fact may also be responsible for the negative results of skin tests.

BAT is based on the exposure of specific antigens against sIgE bound to FcεRI receptors on the surface of basophils. The antigen-antibody specific binding induces the degranulation of the basophils and constituent molecules in the membrane of the basophil granules like CD63 or CD203 are expressed in the cell membrane and can be detected by the antibodies used in the test. The large number of mast cells present in SM patients can reduce the presence of IgE in the membrane of basophils. In fact, BAT has shown good results in patients with HVA without mastocytosis in the same conditions and with the same dose of allergen [10]. Different methods can be used to identify basophils in whole blood by flow cytometry (IgE, CCR3, CD203c/CD123/HLA-DR, CD45/CD123/HLA-DR....). Interestingly, the studies that show little or no validity for BAT assay in SM patients with negative sIgE recognize the basophils by the Eotaxin-3 receptor (CCR3) [5,6] and probably basophils with low amount of IgE on its surface are selected, explaining the bad results of BAT. Under this assumption, detecting the basophils in BAT with an anti-IgE antibody

would clearly improve the results avoiding false negatives. In our study, a sample was tested in parallel using IgE and CCR3 to identify the basophils and we found lower values using CCR3. Even for the *Polistes* trial the results changed from positive to negative (<5%) when basophils were identified by CCR3. The patient was under venom immunotherapy when these second BATs were done, but this fact didn't influence the results as the tests were performed in parallel, with the same blood sample and at the same time. An adequate selection of the molecule used for the identification of basophils in BAT can be an important parameter to obtain optimal results in patients with MS and HVA. Under these premises, BAT may be an adequate tool to assess patients with MS and systemic reactions due to Hymenoptera venom.

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Conflict of interests

The authors have no conflict of interests to disclose.

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Table 1.

Results of sIgE and BAT. Results of BAT using IgE or CCR3 for the selection of basophils in the assay in Patient 1.

	Patient 1	Patient 2
Total IgE	8.06 KU/l	27.9 KU/l
sIgE <i>Vespulaspp</i>	<0.01 KU/l	0.08 KU/l
sIgE <i>PolistesDominulus</i>	0.08 KU/l	0.09 KU/l
sIgE <i>BombusTerrestris</i>	<0.1 KU/l	ND
sIgE <i>ApisMellifera</i>	<0.01 KU/l	0.01 KU/l
r Ves v1	0.01 KU/l	0.02 KU/l
r Ves v5	<0.01 KU/l	0.01 KU/l
r Pol d5	0.05 KU/l	<0.01
r Api m1	<0.1 KU/l	ND
Intradermal tests	Negative	ND
Basal serum tryptase	40.5 µg/l	23.2 µg/l
BAT		
Method for basophil selection	IgE	IgE
BAT negative Control	3.5 %	3.0 %
BAT positive Control (fMLP)	67 %	64 %
BAT <i>Polistes</i>	22 %	21 %
BAT <i>Vespula</i>	41 %	30 %
BAT (6 months later)		
	Patient 1	
Method for basophil selection	IgE	CCR3
BAT negative Control	4.3 %	3.7 %
BAT positive Control (fMLP)	53.5 %	51.2 %
BAT <i>Polistes</i>	40.9 %	4.7 %
BAT <i>Vespula</i>	79.9 %	19.3%

ND: not determined. sIGE: specific IgE. BAT: basophil activation test. fMLP: formyl-methionyl-leucyl-phenylalanine.