

Editorial

Basophil's secrets revealed by flow cytometry

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Basophils are major effector cells of allergic inflammation. These cells originate in the bone marrow from a CD34⁺ progenitor and are usually found in the blood in very low numbers (0.2–1% of total leukocytes) as fully differentiated cells. Human basophils express the high-affinity IgE receptor (FcεRI) which is a main activating receptor for these cells (1). Clustering of IgE bound to the FcεRI leads to basophil activation and to the release of a relatively restricted panel of mediators including histamine, cysteinyl leukotriene C₄ (LTC₄) and cytokines, primarily IL-4 and IL-13 (2).

Understanding cellular and molecular biology of human basophils has long been hampered by the limited number of cells that could be isolated from the blood of either normal or allergic individuals. Studies on basophil function were also limited by the low sensitivity of the assays used to measure mediator release and, thus, by the need for high number of purified cells. Together with the lack of animal sources of basophils or cell lines with characteristics similar to those of the primary cells, these problems were major obstacles for several decades to define the role of human basophils in allergic disorders.

In the early 1990s improved techniques were developed for basophil isolation and purification from peripheral blood. These included positive and negative immunomagnetic selection using microbeads coated with antibodies against major cell surface markers. Although relatively expensive, these techniques were highly effective and relatively fast tools for obtaining pure populations of basophils. At the same time, methodologies for assessing the responses and mechanisms of activation of basophils became more sensitive and specific, allowing *in vitro* studies with a limited number of cells. For example, development of ELISA assays with high sensitivity for

mediators and cytokines led to an increased number of studies on physiological and pharmacological modulation of basophil activation. Improved methodologies to investigate molecular biology and intracellular events associated with basophil activation allowed reproducible results even with very small numbers of purified cells. However, in spite of these technical advances, the role of the basophil *in vivo* in allergy and other diseases remained elusive (3).

As long as studies on basophil activation *in vivo* were based on measurement of mediators released in biological fluids, e.g., blood, urine, nasal, or bronchial lavage fluids, it was difficult to obtain reproducible information. This was mainly because (i) there are no basophil-specific mediators or cytokines, (ii) mediators released by activated basophils, i.e., histamine and LTC₄, are rapidly degraded *in vivo* and standardization of the techniques, even with the most sensitive assays, is difficult to achieve.

In the last 10 years flow cytometry has been increasingly used to investigate activation of human basophils both *in vitro* and *in vivo* (4). Major advantages of the last-generation flow cytometry analyzers are the capacity of identifying cells even in very small percentages of the total population examined (below 1%) and the use of multi-color protocols allowing the simultaneous analysis of several cell surface markers (5). In the last few years, identification of basophil-specific markers and expression of activation-specific molecules on these cells attracted many groups to work in this area (6). In this issue of *Allergy* several papers give a state-of-the-art update of flow cytometry-assisted investigation of human basophils and propose novel applications of this technique in the diagnosis of allergic disorders (7–13).

Initial flow cytometry studies used anti-IgE and anti-FcεRI antibodies to detect resting basophils within peripheral blood leukocyte preparations. However, blood cells other than basophils, i.e., monocytes, eosinophils, and dendritic cells, can express FcεRI or bind IgE under certain circumstances. Moreover, anti-IgE or anti-FcεRI may activate basophils, making the cells unsuitable for subsequent stimulation studies. Earlier reports identified CD203c (neural cell differentiation antigen, E-NPP3), an ectonucleotide pyrophosphatase/phosphodiesterase enzyme, as selectively and constitutively expressed on basophils (14). Cells that are CD203c-positive and HLA-DR-negative are currently identified as quiescent basophils in most flow cytometry studies. The IL-3 receptor α-chain (CD123) has been also proposed as a selective marker for resting basophils (15).

The most popular activation marker for basophils is CD63, a member of the tetraspanin family barely detectable on resting cells and exposed on the cell surface upon degranulation (16–18). The article by Ebo et al. in this issue provides a highly informative and updated review on resting and activation markers of basophils and a balanced opinion of current clinical and experimental applications (8).

Diagnostic test for allergic disorders is currently the main area of research in which flow cytometry-assisted techniques are employed. It is a common opinion among clinical allergists that there are still major 'dark zones' in the diagnosis of conditions such as drug and food allergy. The basophil activation test, based on the expression of CD63, is extensively being used to assess drug-induced activation of basophils *in vitro*. This test offers significant advantages over the *in vivo* measurement of basophil-derived mediators, gives information on both anaphylactic and anaphylactoid reactions and may be a safer alternative to challenge test *in vivo*. Several studies have been published on the utility of the basophil activation test in diagnosing allergy to latex (19), neuromuscular blocking agents (20), β-lactam antibiotics (21), and nonsteroidal anti-inflammatory drugs (22, 23). While most of these studies have shown a good specificity of the test, sensitivity remains a major issue and deserves further investigation. Other investigators have examined the role of basophil activation tests in the diagnosis of food (24, 25) and hymenoptera venom allergy (7). At this moment it is fair to say that a real advantage of flow cytometry assay over skin prick test and specific serum IgE determination remains to be demonstrated.

The role of basophil activation, as assessed by CD63 expression, is being investigated in chronic idiopathic urticaria (CIU). A subset of patients with CIU has an autoimmune disease characterized by the presence of serum antibodies anti-FcεRI or, less commonly, anti-IgE. These autoantibodies are able to induce basophil and mast cell activation both *in vitro* and *in vivo*. The diagnosis of autoimmune CIU is based on autologous serum skin test (ASST). However, ASST cannot be

performed in patients that require continuous antihistamine treatment or have marked dermatographism. Moreover, ASST is not fully standardized and the results are not really quantitative. The observation that sera from patients with autoimmune CIU induced histamine release *in vitro* from basophils isolated from either normal or atopic donors (26) led to the development of a histamine release test that has been validated by a number of papers including one in this issue (11, 27). Another paper in this issue reports the validity of a tricolor flow cytometry, based on CD63 expression, to detect functional autoantibodies in the serum of patients with CIU and positive ASST (10). The flow cytometry test gave results comparable to ASST and was also useful for monitoring clinical response to treatment with cyclosporin A. Thus, currently available data indicate that basophil activation *in vitro*, as determined by either histamine release or CD63 expression, may be useful to confirm ASST results or to diagnose autoimmune CIU in patients in which ASST cannot be performed.

Flow cytometry continues to provide information on novel leukocyte differentiation antigens expressed on human basophils. As new antibodies are developed by the scientific community, they are tested on basophils from either normal individuals or patients with allergic diseases or hematologic malignancies (28). These studies are aimed to identify markers that can be used for better discrimination of basophils from other cells or as more sensitive indicators of cell activation. The article by Florian et al. in this issue is a very good example of such studies (9). Using a large series of monoclonal antibodies, the authors detected clearly distinct patterns of CD expression on basophils and mast cells, thus providing further evidence that these two cells represent distinct lineages within the hematopoietic cell family.

Besides being instrumental for studies on differentiated cells, flow cytometry is extensively used to investigate the ontogeny of human basophils. The nature of the progenitors and the maturation stages of basophils in the bone marrow are still largely unknown. A better knowledge of the pathways leading to basophil differentiation in humans could be very useful to understand early phases in the pathogenesis of allergic diseases. Reimer et al. proposed a novel method to obtain immature basophils from cord blood cells incubated with IL-3 (13). Flow cytometry analysis in this study revealed that FcεRI and CD203c expression is an early event during basophil maturation. These two markers can be used to purify populations of immature basophils suitable for investigating the terminal differentiation of these cells.

Current evidence indicates that flow cytometry-assisted techniques may be a useful tool for both experimental and clinical studies of the functions of basophils in allergic diseases. This test is faster, offers quantitative and reproducible results, and is probably more specific than other tests based on mediator release. However, many technical issues remain to be addressed, including the use

of whole blood vs leukocyte-enriched populations, whether cells should be from normal or atopic donors, and if preincubation with IL-3 should be used. A complete standardization of the procedures used to study human

basophil activation by flow cytometry is mandatory before studies in large patient populations could provide useful information on the role of this test in the allergy clinic.

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