

EDITORIAL

The return of the basophil

The basophil was first described in the peripheral blood by Ehrlich more than one hundred years ago [1]. For much of the time that has elapsed since, this cell type has languished in the shadow of its tissue-resident counterpart: the mast cell. Basophils have been characterized as a rich source of histamine, eicosanoids, cytokines and other mediators, but they are the rarest of leucocytes, normally accounting for less than one percent of those leucocytes found in blood [2,3]. There have long been reports that the numbers of these cells may be increased in the peripheral blood in allergic disease, or that they may migrate into the affected tissues. However, much of the evidence for basophil accumulation in tissues has been circumstantial, and research in this field has come to be neglected. Only recently have appropriate tools become available that allow the reliable detection of basophils in human tissue.

Histochemical staining with basic dyes, the mainstay of basophil detection procedures since the time of Ehrlich, are generally unsuitable for tissues routinely processed with formaldehyde-based fixatives, and do not allow basophils to be distinguished reliably from tissue mast cells. The dye is taken up by proteoglycans in the secretory granules of both of these cell types, and it is a major challenge to discern the subtle differences in the relative size and numbers of granules, or in the shape of the nucleus. At the ultrastructural level, the larger size of basophil granules, and the segmental nucleus of this cell type is more apparent [4], but electron microscope studies covering small areas of tissue are not suitable for generating quantitative data. In the absence of a unique marker for basophils, some workers have employed a double labelling procedure to identify cells strongly expressing the high affinity IgE receptor, but which fail to express the mast cell marker tryptase [5]. The value of such approaches as a means for identifying basophils has been undermined by the recognition not only that various other cell types may express FcεRI [6,7] but also that cells of the peripheral blood (presumably basophils) may sometimes express quite high levels of tryptase [8].

Advances in the understanding of the biology of human mast cells occurred in the late 1980s and in the 1990s with the development of monoclonal antibodies specific for tryptase [9,10], other proteases of the mast cell granule [9,11,12], and for the membrane marker c-kit [13]. A basophil-specific antibody was produced by Bodger et al. as long ago as 1987. This antibody, termed Bsp-1, binds in flow cytometry to a 45-kDa cell surface protein expressed by basophils [14] and a proportion of KU812 cells (a line with basophilic properties [15]), but is generally unsuitable for the immunohistochemical identification of basophils in tissues. Another antibody, named 97A6, is directed against a cell surface antigen that is reported to be expressed more abundantly following basophil activation [16]. However, this antigen is expressed to some extent also by mast cells and its potential as a marker for basophils remains to be clarified. Two monoclonal antibodies that recognize granule proteins specific

for basophils are now showing great promise as a means for the identification of this cell type. These antibodies, termed 2D7 [17] and BB1 [18], bind to proteins of unknown function that are secreted upon degranulation.

Antibody 2D7 binds to a protein of 72–76 kDa [17] that is produced at an early stage in basophil development from bone marrow progenitors [19]. BB1 antibody recognizes a highly basic protein, termed basogranulin, that appears to exist as a large macromolecular complex of approximately 5000 kDa or more, and is secreted along with histamine upon cell stimulation [18,20]. The development of specific immunoassays for basogranulin and the 2D7 antigen, and elucidation of their mediator roles, could one day provide important clues as to what may be the unique contribution of basophils in allergic disease. In the meantime, BB1 and 2D7 are providing a means for the reliable identification of basophils in tissues for the first time and are allowing relative numbers of this cell type to be determined in allergic conditions.

In the absence of allergen exposure, relatively few basophils appear to be present in the tissues of allergic subjects. Immunostaining with BB1 antibody has revealed that basophils are rare in nasal biopsies taken from subjects with seasonal allergic rhinitis out of season [21]. In the lower airways, basophils detected by the same technique are slightly more numerous in bronchial biopsies from asthmatic subjects than in those from control subjects, but they are much less common than either mast cells or eosinophils [22]. In unstimulated skin of atopic subjects, basophils are rarely, if ever, observed [23]. Following experimental allergen challenge, basophil numbers may increase dramatically in all of these tissues.

Intranasal administration of allergen can induce the rapid influx of basophils into the nasal mucosa of rhinitic subjects [21]. This response can be evident as early as one hour after allergen challenge, and significant basophilia can last for at least a week afterwards. There have been close parallels in asthmatic subjects, with increases in the basophil numbers in bronchial biopsies observed in response to inhalation challenge [22,24], and a substantial proportion of these cells have been noted to possess IL-4 [24]. In the skin also, allergen-provoked reactions have been found to be associated with basophil infiltration into the inflamed area after challenge [22,25]. Diffuse staining of extracellular material reacting with both 2D7 and BB1 has been taken as indicative of basophil degranulation at these sites.

The kinetics of basophil recruitment following allergen challenge is quite different from that of eosinophils or neutrophils, suggesting different processes involved in cell recruitment. Thus, for example, Ying and colleagues have reported that basophil numbers are maximal at 24 h when eosinophil numbers have declined from their 6-h maximum [23]. In that study there was a clear association between the degree of eosinophil infiltration at 6 h and eotaxin expression in the tissues,

and at 24 h with eotaxin-2 and monocyte chemotactic protein 4 (MCP-4) expression; whereas expression of none of these CC chemokines, nor of RANTES or MCP-3, was related to the numbers of basophils at the 24-h peak. In the model of Fahy et al. [26], in which human skin has been grafted onto severe combined immunodeficiency (SCID) mice reconstituted with human mononuclear cells, injection of eotaxin or RANTES, but not monocyte-derived chemokine (MDC) or IP-10, has been found to stimulate the influx of BB1-reactive cells [26]. Processes of basophil recruitment are likely to be complex, and could differ between clinical conditions and tissue compartments.

Increased numbers of basophils in the affected tissue following experimental allergen challenge have now been reported in several studies. The effect of chronic natural allergen exposure on numbers of tissue basophils, or the effect of treatment, has not hitherto been reported. In a recent issue of *Clinical and Experimental Allergy*, Wilson et al. describe the results of a study in which basophils were enumerated in nasal tissue collected from subjects with seasonal allergic rhinitis outside the pollen season and from the same individuals two years later at the height of the pollen season [27]. Over this period, subjects received a course of allergen-specific immunotherapy or placebo. In untreated subjects, basophils as detected immunohistochemically with 2D7 antibody were found to be more numerous in both the submucosa and epithelium in tissues collected during the pollen season. In keeping with previous studies, seasonal increases in numbers of eosinophils and mast cells, but not neutrophils, were noted in biopsy tissues. Basophil infiltration into the epithelium during the pollen season appeared to be reduced by allergen-specific immunotherapy, an observation made also for eosinophils. This study thus provides further evidence for a role for basophils in the pathogenesis of seasonal allergic rhinitis, and the authors suggest that immunotherapy may work, at least in part, by reducing the recruitment of basophils to the nasal tissues.

This is an exciting time for basophil research. We now have confirmation that appreciable numbers of these cells can be recruited into the tissues following experimental or natural chronic exposure to allergen, and that immunotherapy may be associated with reduced numbers. There is still much to learn about the underlying processes of basophil accumulation and how recruitment and activation may be modulated by immunotherapy and other treatments. The availability of the new basophil-specific antibodies is opening up new avenues for investigation, and should allow a better evaluation of the true roles of these neglected cells in allergic disease.

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