WHEN IS A MOUSE BASOPHIL NOT A BASOPHIL?

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ABSTRACT

The identification and characterization of mouse basophils have historically been hampered by the extreme rarity of this cell type. Virtually no photomicrographs of hematologically stained (e.g., Wright-Giemsa) examples of mouse basophils exist in the literature. However, four recent papers in the last two years have used flow cytometry and a defined set of cell surface markers to identify and subsequently isolate mouse “basophils”, including the publication of stained cytospin preparations of these cells. Surprisingly, a reevaluation of the data from all four of the papers revealed several issues of concern which suggest that the cells under study are not necessarily basophils. Nonetheless, we propose that these manuscripts do provide the foundation for a reevaluation of the defining characteristics of a basophil and/or provide support for the provocative conclusion that a new previously overlooked leukocyte subtype has been identified. The purpose of this commentary is to revisit these previously published papers, highlight the relevant issues, and provide a different perspective in the hope of developing a consensus within the research community as to the true identity of the “basophils” described in these studies.
INTRODUCTION

The roles of the different granulocyte subtypes in allergy and asthma have been the subject of continued study and debate. Reports from both the clinical literature \(^1\) and studies of mouse models \(^2\)-\(^5\) have recently focused particular attention on basophils as potentially critical cells in the development of inflammatory diseases such as asthma. The ability to identify unambiguously human basophils, and subsequently isolate and characterize these granulocytes, has uniquely enabled physician-scientists to develop and test hypotheses linking these cells with pathological changes occurring in patients. Unfortunately, similar advantages have been by and large out of the reach of investigators using mouse models of disease. Unlike humans, mouse basophils are exceptionally rare \(^6\) and, until recently, only a single photomicrographic example of these leukocytes was reported in the literature \(^7\). As a consequence, electron microscopic identification has generally been substituted as a criterion for identification of basophils in the mouse (see for example \(^8\)). However, during the last two years, four papers utilizing mouse models \(^2\)-\(^5\) employed flow cytometry and a defined set of cell surface markers to identify mouse “basophils” and provide photomicroscopic evidence of their appearance following hematological staining (e.g., using DiffQuik (Fisher)). The provocative character and clinical relevance of the conclusions in these papers are significant and warrant careful reexamination of the data presented, particularly in regards to the identification of the leukocytes under study as “basophils”. This is especially true given the inter-dependence and self-fulfilling character of these reports. That is, the second of the manuscripts published \(^3\) cites the first paper \(^5\) to confirm the identification of the leukocytes under study as “basophils” and the third \(^4\) and fourth \(^2\) papers, in turn, cite the previous two as support for their identity.
IMMUNOLOGY vs. HEMATOLOGY

The manuscripts by Voehringer and colleagues 5, Min et al., 3, Gessner and colleagues 4 each assessed the cellular source of IL-4 expression in naive mice as well as animals following infection with the intestinal nematode *Nippostrongylus brasiliensis* using unique GFP “knock-in” mice. In contrast, Mukai and colleagues 2 examined the role of “basophils” in the development of IgE-mediated chronic allergic inflammation. Despite the use of different models (i.e., parasite infected animals vs. non-infected animals) and strains of mice (BALB/cJ vs. C57BL/6J), these papers share three important commonalities: (i) Through the use of flow cytometry and antibodies specific for unique cell surface markers, the authors describe cells with a surface immuno-phenotype that they characterized as representing a “basophil” (i.e., FSClo/SSClo, Thy-1.2+, CD69+, CD49b(DX-5)+, FceRI+, 2B4+, CD11bnull, CD24+, CD19+, CD80+, CD14+, CD23+, Ly49C+, CD122+, CD11c+, Gr-1-, α4 and β7-integrin-, c-kit-, NK1.1-, CD3+, B220+, γδTCR-, αβTCR-). The basis for this definition initially arose from studies of human leukocytes (see for example 9,10) and has continued to evolve in the mouse (see for example 11,12). Ultimately, the definition is fundamentally simplistic: granulocytes with lower than expected scatter characteristics in flow cytometry that are FceRI+, CD49b(DX-5)+, and negative for a host of lineage markers that rule out other leukocyte subtypes; (ii) The “basophils” in these studies are relatively prevalent leukocytes. In three of the studies 2-4, the authors noted that the identified leukocytes were ~1% of nucleated marrow cells and in the fourth study 5 the identified leukocytes comprised more than 2% of the total IL-4 expressing cells accumulating in the lungs of mice following *N. brasiliensis* infection; (iii) Each of the studies used cell sorting followed by preparation of cytopins and hematological staining to confirm the identity of the leukocytes as “basophils”. Figure 1 reproduces the photomicrographs from each of these papers as they appeared in the original publications. The stained cells in each panel display identical morphologies: the nuclei are polymorphic and the cytoplasmic regions are slightly basophilic and remarkably devoid of any granulation (i.e., stained as well as unstained granules). Herein lies the problem - these are neither the morphology nor the staining characteristics of basophils! Panels A and B of Figure 2 contain representative photomicrographs of basophils we have collected from Wright stained blood films of mice. These cells display the characteristic features
associated with basophils: leukocytes similar in size with other blood granulocytes (i.e.,
eosinophils and neutrophils) that, however, contain intensely metachromatic stained granules
which are asymmetrically distributed throughout the cytoplasm and a lobulate nucleus that has
comparatively weak staining chromatin. Interestingly, the cited manuscripts suggest that the
leukocytes that were identified by flow cytometry have the correct hematological morphology
through a series of references which ultimately lead to the single paper in the literature that had
previously presented a photomicrograph (unfortunately in black and white) of a mouse basophil
7. For example, Mukai, et al. 2, references 12 which, in turn, ultimately leads to Urbina and
colleagues 7. This singular photomicrograph is reproduced here and is shown in Figure 2(C).

The hematological staining character of this leukocyte is remarkably similar to the other
representative mouse basophils presented in Figure 2, all of which share the hallmark
morphologies that define a basophil. In particular, Urbina and colleagues 7 note in their 1981
manuscript “The mouse basophils differ from those of other species in that they have relatively
few, loosely packed granules of unequal size. Still, these granules appear intensely azurophil
and characteristically metachromatic in the blood smears.” Surprisingly, the “basophils”
identified in the four cited manuscripts under discussion (Figure 1) bear little resemblance to
any of the representative mouse basophils in Figure 2, including the photograph from Urbina
and colleagues. The photomicrographs of Figure 2 also include a comparison of mouse
basophils with a human basophil from a peripheral blood film (Figure 2(D)). This comparison
clearly shows the commonality of this staining pattern between species and the evolutionary
conservation of this leukocyte.

In addition to lacking the appropriate morphology and staining characteristics, the “basophils”
identified in the four cited manuscripts are also far too prevalent. Mouse basophils are
extraordinarily rare cells (as witnessed by the virtual lack of photo-documentation in the
literature) such that in our combined 50 years of mouse hematological studies we have identified
only a total of 5 mouse basophils with the correct morphology and staining pattern. Indeed, we
recently scanned and typed by light microscopy 10,000 leukocytes from peripheral blood films
as well as bone marrow brush smears of BALB/cJ and C57BL/6J mice and were unable to
identify a single cell with the appropriate staining characteristics; this despite a projected (with
99.9% confidence) identification of 70 basophils from 10,000 leukocytes if they represented ~1% of nucleated cells.

There is no reason to doubt that each of the cited manuscripts have identified a leukocyte with the suggested inventory of cell surface markers. It is also clear from these studies that this leukocyte is prevalent, representing ~1% of marrow mononuclear cells. Furthermore, the hematological staining characteristics described in these manuscripts are consistent with one another and in each case represent the leukocytes with the described cell surface marker profile. However, the identification of a leukocyte as a basophil is not dependent on assorted “CD” markers present or absent in flow cytometry. Instead, it is a consequence of a cell’s appearance and characteristic morphology following hematological staining. By these distinguishing hematological features, the leukocytes identified in the cited manuscripts are not basophils.
WHAT’S IN A NAME?

It is possible that the discrepancy between the 1981 paper of Urbina et al., 7 and the cited manuscripts under discussion (i.e., regarding the staining morphology characteristic of mouse basophils) occurred over time as succeeding references incrementally misinterpreted statements made by the original authors. Specifically, the statement by Urbina and colleagues “The mouse basophils differ from those of other species ...” appears to have evolved to - mouse basophils don’t look like human basophils. In addition, the statement “they (basophils) have relatively few, loosely packed granules of unequal size” is now interpreted as - mouse basophils have no granules. However, and irrespective of the ultimate source of this confusion, the leukocytes identified in the four cited manuscripts do not display the classical morphology and staining characteristic of basophils as first described in 7 and now confirmed in this perspective (Figure 2).

So ... where does this leave us? We suggest that three explanations exist for the observations noted in this perspective. First, the leukocytes in the cited manuscripts and those identified here as basophils may represent the same cell at different points of maturation. Although possible, the identification of leukocytes with similar morphology in both bone marrow and peripheral tissues would suggest that the cells in the cited manuscripts do not substantially change as they mature and are therefore different from the basophils shown here. The second possibility is that basophils in rodents include two morphologically distinct subtypes. One that is classical in appearance, rare, and has a yet-to-be-defined cell surface marker profile vs. leukocytes with a unique surface immuno-phenotype which are less distinct in appearance but nonetheless more prevalent. The lack of such a described dichotomy in other mammalian species, including humans, would suggest that its unique appearance in the mouse is unlikely. However, this issue has certainly not been fully explored and it is also possible that these two apparently different and distinct subtypes are simply morphological variations of a single class of leukocytes. Moreover, the possible presence of “basophils” in humans with the characteristics noted in the four cited manuscripts has not been investigated. The final possibility is that these reports have
identified a distinct and previously underappreciated leukocyte. That is, this new cell type shares some characteristics of basophils (e.g., the cell surface marker profile) but is a non-granulated polymorphonuclear leukocyte subtype distinct from the classical metachromatically staining granulated basophil. We suggest that although provocative, this conclusion has merit and may not easily be dismissed based on available data.
FUTURE DIRECTIONS AND POSSIBILITIES

The resolution of this paradox and the unambiguous identification of the cells in the cited manuscripts are critical to the integration of data from asthma patients and mouse models of disease. Specifically, is there a need and/or consensus in the research community to modify the definition of a basophil to include the morphologically distinct leukocytes in the four cited manuscripts under study? Alternatively, are these cells a potentially new leukocyte subtype and is this a mouse-specific phenomenon or do these cells also exist in humans? We believe that each of these are important questions for the hematological as well as immunological communities. Furthermore, we also believe that future studies are required to resolve these issues or, at a minimum, are needed to develop a consensus. Regardless of the eventual outcome of this dialogue, if past experiences are any indication of future events, these studies will also no doubt create more questions than they answer.
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FIGURE LEGENDS

Figure 1. Photomicrographs of leukocytes characterized as “basophils” in four recently published manuscripts. (A) FcεRI+ IL-4 expressing FSClo/SSClo cells recovered from the lungs of *N. brasiliensis* infected mice (BALB/c) stained with Wright-Giemsa (Figure 1 in 5). (B) IL-4 expressing DX5 (CD49b)+ non-CD4+ T cells from the liver of *N. brasiliensis* infected mice (BALB/c) stained with Wright-Giemsa (Figure 3 in 3). (C) FcεRI+ IL-4 expressing CCR3− cells recovered from the blood of naive mice (BALB/c) stained with Wright-Giemsa (Figure 3 in 4). (D) FcεRI+/DX5+ bone marrow cells recovered from normal C57BL/6J mice stained with Giemsa (Figure 5 in 2).

Figure 2. Photomicrographs of peripheral blood leukocytes displaying the classical staining and morphology of basophils. (A) A basophil from a Wright-Leishman stained mouse (strain unknown) peripheral blood film. (B) Wright-Giemsa stained basophil from a peripheral blood film of a mouse on a C57BL/6J background.14 (C) The single previous photomicrograph of a mouse basophil appearing in a paper by Urbina et al.7. (D) A human basophil from a Wright-Giemsa stained peripheral blood film. Scale bar = 10µm. The photographs were collected using a Zeiss Axiophot microscope (Plan-NeoFluar 63x/1.25mm) and a AxioCam MRc5 digital camera. Adobe Photoshop was used to assemble the figure, however, no enhancements or manipulations of the images were made.
REFERENCES


J. Lee Figures

Figure 1
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Figure 2
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