Basophils Are Back!

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In this issue of *Immunity*, Tsujimura et al. (2008) report that the release of platelet-activating factor by basophils stimulated with immunoglobulin G1 (IgG1)-antigen immune complexes contributes substantially to the expression of an IgG1-dependent alternative pathway of systemic anaphylaxis in mice.

Not so long ago, some investigators thought that mice might entirely lack basophils [reviewed in (Urbina et al., 1981)]. Tsujimura et al. (2008) have now helped highlight the importance of mouse basophils by providing strong evidence that these cells can contribute substantially to fatal systemic anaphylaxis.

The least prevalent of circulating granulocytes, basophils usually account for less than 1% of blood leukocytes in normal humans or mice (Marone et al., 2002; Galli et al., 2005). Basophils share several striking similarities with mast cells, including expression of the high-affinity receptor for immunoglobulin E (IgE) (FcεRI), the ability to secrete—upon stimulation with IgE and specific antigen—a similar, but not identical, spectrum of mediators and cytokines, and the presence of cytoplasmic granules that stain metachromatically with certain basic dyes (Marone et al., 2002; Galli et al., 2005).

However, mature mast cells are not normally present in the blood, but instead enter tissues as immature progenitors that undergo the last stages of their development in those anatomical sites where they ultimately will reside (Marone et al., 2002; Galli et al., 2005). In contrast, basophils typically mature in hematopoietic tissues and then, like neutrophils and eosinophils, circulate in the blood until they are eliminated or recruited into tissues (Marone et al., 2002; Galli et al., 2005).

Among the many proposed functions of basophils in health and disease (Marone et al., 2002; Galli et al., 2005), there has been particular interest in the extent to which basophils can contribute to systemic anaphylaxis (Bochner and Lichtenstein, 1991; Galli, 2005). In humans and other mammals, systemic anaphylaxis (or, simply, anaphylaxis) can be induced when certain unfortunate subjects previously sensitized to an antigen are later exposed to even very small amounts of that antigen, which is rapidly followed by the development of severe and sometimes fatal pathophysiological responses (Bochner and Lichtenstein, 1991; Galli, 2005). Because anaphylaxis can be induced by antigens derived from intrinsically innocuous substances, such as components of peanuts or other foods, anaphylaxis is arguably the most grotesque example of a pathological imbalance between the cost and benefit of an acquired immune response.

Several lines of evidence suggest that both mast cells and basophils contribute to systemic anaphylaxis in humans (Bochner and Lichtenstein, 1991; Marone et al., 2002; Galli, 2005; Galli et al., 2005). Clinical observations indicate that the major antibody isotype responsible for anaphylaxis in humans is IgE, document that signs and symptoms of anaphylaxis can be observed within seconds of intravenous exposure to specific antigen, and show that anaphylaxis can be associated with evidence of basophil, as well as mast cell, activation (Bochner and Lichtenstein, 1991; Marone et al., 2002; Galli, 2005; Galli et al., 2005). Both mast cells and basophils can rapidly secrete histamine, lipid mediators, and other biologically active products upon activation with IgE and specific antigen. Further, given the cells' anatomic distribution, blood-borne antigens will first encounter basophils before gaining access to tissues, where they would then encounter mast cells. However, the role of basophils in systemic anaphylaxis in the mouse has remained an open question.

Why is that? First, mouse basophils have been difficult to study. They can be identified on the basis of their ultrastructural features (Dvorak et al., 1982), but this is a problematic way to attempt to analyze their functions in vivo. Second, in contrast to mast cells, whose functions can be analyzed in mice that genetically lack mast cells but which can be engrafted with normal or genetically altered mast cells (Galli et al., 2005), no mutant mice have been reported that selectively lack basophils. Finally, although it has been known for many years that either IgE or IgG1 antibodies can mediate antigen-specific fatal anaphylaxis in mice and that fatal anaphylaxis can be elicited in mice that virtually lack mast cells (or that lack IgE or the IgE-binding α chain of FcεRI) [reviewed in (Miyajima et al., 1997; Tsujimura et al., 2008)], evidence has been presented indicating that the macrophage is the main source of platelet-activating factor (PAF) and perhaps other mediators that are responsible for IgG1-dependent systemic anaphylaxis in mice [reviewed in (Finkelman, 2007)].

In this issue of *Immunity*, Tsujimura et al. (2008) now report several lines of evidence strongly implicating basophils as an important source of PAF, and as the cell type most responsible for a fatal outcome, in IgG1-mediated anaphylaxis in mice. When either normal (C57BL/6) or genetically mast-cell-deficient C57BL/6-$Kit^{W\text{-}sh/W\text{-}sh}$ mice were passively sensitized with Penicilin V (PenV)-specific IgG1 monoclonal antibodies (mAbs) and then challenged with PenV conjugated to bovine serum albumin (PenV-BSA) intravenously, both types of mice developed systemic anaphylaxis (as measured by a drop in rectal temperature). However, C57BL/6-$Kit^{W\text{-}sh/W\text{-}sh}$ mast-cell-deficient mice had a less severe drop in temperature than did control mice. These results

are in accord with the findings that the tachycardia and changes in pulmonary function, as well as the mortality, associated with a different model of IgG1-dependent passive anaphylaxis were less dramatic in WBB6F$_1$-*Kit*$^{W/W\text{-}v}$ mast-cell-deficient mice than in the corresponding wild-type mice (Miyajima et al., 1997).

Among several hematopoietic lineages assessed by flow cytometry, basophils displayed the highest expression of IgG1- and FcγRII- or FcγRIII-dependent surface capture of PenV-BSA. Moreover, in vivo depletion of basophils (to 10%–20% of normal numbers) in C57BL/6 mice with a mAb (Ba103) to CD200R3 greatly suppressed anaphylactic shock induced either by anti-PenV IgG1 and PenV or by another IgG1 antibody, anti-2,4,6-trinitrophenol (TNP) IgG1, and TNP-BSA, whereas basophil depletion did not markedly influence IgE-mediated passive anaphylaxis. Suppression of IgG1-mediated anaphylaxis also was observed in Ba103-treated mast-cell-deficient mice. Hence, Tsujimura et al. (2008) concluded that basophils play a critical role in IgG1-, but not IgE-, mediated passive systemic anaphylaxis.

Treatment with the PAF antagonist, CV6209, prior to IgG1 and antigen challenge almost completely blocked IgG1-mediated anaphylaxis in both wild-type and mast-cell-deficient mice. In contrast, inhibition of histamine and serotonin with cyproheptadine, which substantially reduced the drop in temperature associated with IgE-dependent anaphylaxis, had little or no effect on IgG1-mediated anaphylaxis. The authors concluded from this and other evidence that PAF, not histamine or serotonin, was a critical mediator of IgG1-mediated anaphylaxis, and basophils were the most likely source of PAF.

To assess the role of basophils in active systemic anaphylaxis (in which both IgE and IgG1 antibodies are involved), mice were immunized with PenV-OVA or TNP-OVA and challenged intravenously 14 days later with PenV-BSA or TNP-BSA. Strikingly, pretreatment with Ba103 to deplete basophils 1 day before antigen challenge protected mast-cell-deficient mice, but not wild-type mice, from death due to active anaphylaxis. These findings support the conclusion that both basophils and mast cells played important but distinct roles in these models of active anaphylaxis, probably reflecting their involvement in the IgG1- (basophils) versus IgE- (mast cells) dependent components of the response.

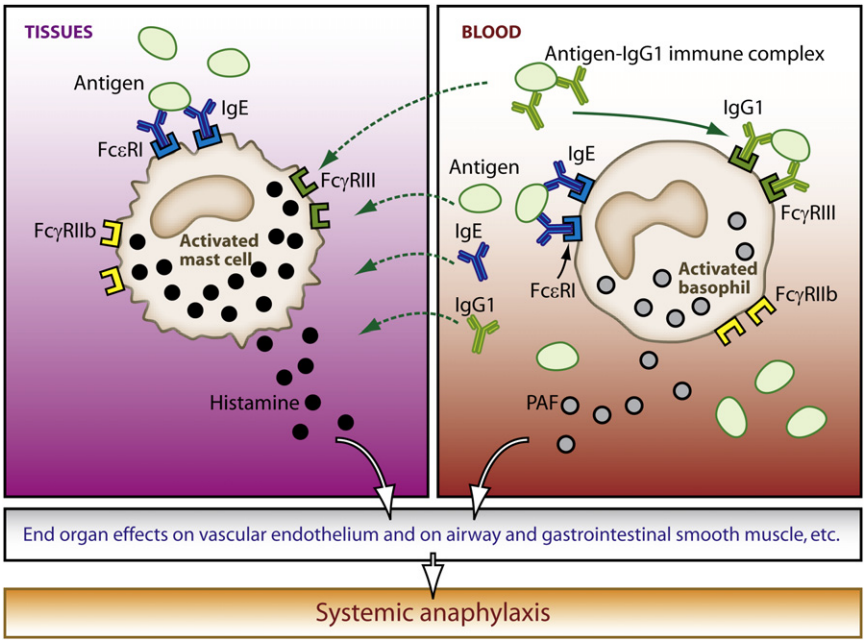

Figure 1. Contributions of Mast Cells and Basophils to Systemic Anaphylaxis in Mice
In sensitized mice that have been injected with specific antigen (Ag) invtravenously, Ag can be recognized by basophils that have bound Ag-specific IgE to surface FcεRI, or can form immune complexes with Ag-specific IgG1 antibodies. By diffusion through blood-vessel walls lined by vascular endothelial cells, Ag (as well as circulating IgE and IgG1 antibodies and Ag-IgG1 immune complexes) also can gain access to mast cells in tissues, especially at sites of enhanced vascular permeability. Ag-specific IgE and IgG1 also can be produced locally in the tissues. Both basophils and mast cells can be activated to secrete mediators when surface FcεRI is aggregated by the binding of FcεRI-bound IgE to bi- or multivalent Ag. Each cell type also can be activated when surface FcγRIII binds immune complexes of IgG1 and specific Ag. Negative regulation of mast cell or basophil secretion of mediators can occur if Ag bound to IgG1 (or to both IgG1 and IgE) antibodies coligates FcγRIII (or FcεRI) and the inhibitory receptor, FcγRIIb. In IgG1-dependent passive systemic anaphylaxis, platelet-activating factor (PAF) derived from basophils is a major mediator of the pathophysiology.

Using the terminology introduced by Finkelman and colleagues (Finkelman, 2007), Tsujimura et al. (2008) proposed that two distinct pathways can result in systemic anaphylaxis in mice: a classical pathway consisting of antigen, IgE, FcεRI, mast cells, and histamine and an alternative pathway consisting of IgG1-antigen immune complexes, FcγRIII, basophils, and PAF (Figure 1).

Does this study close the book on the roles of basophils versus mast cells versus macrophages or other cell types in anaphylaxis in the mouse? Not entirely. As always, the conclusions of this study are based on the models used, and these relied heavily on the specificity and efficacy of Ba103 and the other antibodies that were used to deplete specific hematopoietic cell types. As noted by Tsujimura et al. (2008), Ba103 identifies CD200R3 on both basophils and mast cells (Obata et al., 2007). Even though treatment with Ba103 did not substantially influence the magnitude of IgE-mediated local cutaneous or systemic anaphylaxis in normal mice (Obata et al., 2007), nor did short-term treatment with Ba103 deplete skin or peritoneal mast cell populations in vivo (Obata et al., 2007), the authors have not formally excluded the possibility that Ba103 might influence IgG1-dependent anaphylaxis in part through effects on mast cell function. For example, the enhanced vascular permeability associated with anaphylaxis might increase exposure of tissue mast cells, which also express FcγRIII (as well as the inhibitory receptor, FcγRIIb), to IgG1-antigen immune complexes (Figure 1). However, IgG1-dependent passive systemic anaphylaxis, as well as fatal active anaphylaxis, can be observed in the virtual absence of mast cells (Miyajima et al., 1997; Tsujimura et al., 2008), and treatment with Ba103 markedly diminished the severity of each of these models of anaphylaxis in

mast-cell-deficient mice (Tsujimura et al., 2008); these facts strongly support the conclusion that basophils can contribute importantly to the pathology of both passive IgG1-dependent and fatal active anaphylaxis.

Should we now dismiss the possibility that macrophages also can contribute importantly to IgG1-dependent anaphylaxis? Probably not. Tsujimura et al. (2008) used mice of a single strain background (C57BL/6) to investigate models of passive or active anaphylaxis to PenV or TNP-BSA; in each case, mice were challenged with antigen intravenously. It is possible that macrophages (as well as additional cell types that can produce PAF or other bioactive mediators) may contribute to the pathology observed in other models of anaphylaxis, such as those studied by Finkelman and colleagues [reviewed in (Finkelman, 2007)], and/or in models of anaphylaxis induced in other strains of mice.

Finally, it is obvious that the conclusions of Tsujimura et al. (2008) apply to mice (specifically, C57BL/6 mice), and many questions remain about the extent to which findings in this or other studies of mouse models of anaphylaxis can help to elucidate the pathology of anaphylaxis in humans (Bochner and Lichtenstein, 1991; Miyajima et al., 1997; Galli, 2005; Finkelman, 2007; Tsujimura et al., 2008). Nevertheless, the mouse basophil is making progress: from a cell whose existence was disputed to center stage in the efforts to understand the complex and redundant mechanisms responsible for the most devastating of acute allergic disorders, anaphylaxis.

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