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XBP1, a novel regulator of the eosinophil lineage

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Abstract

Targeted deletion of the transcription factor XBP1 in hematopoietic stem cells selectively prevents eosinophil maturation in the bone marrow without affecting other immune lineages.

The X-box-binding protein 1 (XBP1) is essential for the development of cells with a secretory phenotype presumably by modulating the unfolded protein response (UPR) during ongoing endoplasmic reticulum (ER) stress¹. A functional role for XBP1 in hematopoietic stem cell differentiation has not been described. In this issue of *Nature Immunology*, Bettigole *et al.* demonstrates that deletion of the *Xbp1* gene in the hematopoietic lineage selectively and potently prevents the maturation of eosinophil progenitors (EoPs) without affecting other lineages in the bone marrow (Fig. 1)². This gene targeting has unexpectedly created a novel strain of mice with a highly specific defect in eosinophilopoiesis and a complete lack of mature, circulating peripheral blood eosinophils. The authors used a *Vav1*-Cre mouse to specifically delete *Xbp1* in multi-lineage hematopoietic progenitors that give rise to myeloid-granulocytes (eosinophils, neutrophils, basophils, monocytes and mast cells) and lymphoid/lymphocytes (B cells, T cells and natural killer cells) (Fig. 1). They show that XBP1 is highly and selectively activated during eosinophil commitment from granulocyte-monocyte progenitors (GMPs) and in its absence, EoPs exhibit defective protein folding, attenuated granule formation, leading to differentiation arrest and cell death.

Since its discovery in 1879, the eosinophil has remained an intriguing but generally poorly understood granulocyte. Because of its apparent involvement in innate and adoptive immunity and presence in a variety of increasingly common human diseases including parasitic infection, allergy, hypereosinophilic syndrome and esophagitis, the eosinophil has engendered considerable recent research attention. Eosinophils, along with neutrophils and basophils, constitute the three principal types of blood granulocytes. They are distinguishable by their appearance after Wright's stain. At the earliest stages of eosinophil differentiation, cytoplasmic granule proteins appear. These are released at the sites of inflammation and play a crucial role in the killing of microorganisms and parasites as well as in response to allergen. Each granulocyte type synthesizes distinct granule proteins (cationic proteins, enzymes, cytokines and chemokines) and differentiates through the coordinated activity of multiple, overlapping transcription factors (for example, GATA-1, GATA-2, PU.1 and C/EBP α) in response to signaling from distinct cytokines (including

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interleukin 3 (IL-3), IL-4, IL-5, GM-CSF, M-CSF and G-CSF). To date, no single master gene has been identified that controls the commitment of GMPs to EoPs. XBP1 is the first transcription factor that uniquely defines eosinophil development from that of other granulocytes and plays an indispensable role in the terminal differentiation of the eosinophil lineage (Fig. 1).

In highly secretory cells (such as granulocytes, plasma cells, paneth and pancreatic acinar cells) with extensive ER networks, the folding of nascent proteins is extremely error-prone. Thus, these cells must cope with the continuous burden of misfolded or unfolded proteins and substantial ER stress. This dilemma is resolved by the timely activation of adaptive UPR pathways to reduce protein influx into the ER (ER stress response) and activate degradation pathways to dispose of cytotoxic proteins. IRE1-XBP1 is a component of three mammalian UPR pathways also found in the eosinophil lineage². Upon ER stress, IRE1 translocates into the nucleus, binds ER stress response elements (ERSE) and induces the transcription of *Xbp1* and chaperones that are involved in ER-associated protein degradation¹. During maturation, eosinophil progenitors are abruptly faced with the physiologic demands of granule protein production, forcing these cells to rapidly adapt to escalating ER stress with enhanced protein-folding capacity. Not surprisingly, *Xbp1*-null EoPs are extremely sensitive to ER stress and attempt to compensate for the absence of XBP1 by upregulating PERK-AFT4 expression², an alternative UPR pathway. While cells lacking XBP1 are capable of producing eosinophil-specific granule proteins and some immature granule structures², the aggregate UPR response is inadequate to restore ER homeostasis, leading to the downregulation of GATA-1 activity, reduced transcription of eosinophil granule genes and eventual cell death.

The IRE1-XBP1 pathway is the most highly conserved ER stress sensor in eukaryotes, and mice lacking IRE1 or XBP1 are embryonic lethal at an early developmental stage^{3,4}. Most immune cells, however, do not require XBP1 to survive developmental ER stress. Plasma cells lacking XBP1 are found at normal numbers but cell maturation and immunoglobulin production are reduced⁵. In addition, total bone marrow cellularity and splenic immune cell numbers are unaffected by XBP1 deficiency although a minor decrease in the number of splenic dendritic cells (DCs) is present². Thus, with the exception of eosinophils, XBP1 deficiency is generally well tolerated across the rest of the immune system. Presumably this sensitivity reflects the unique composition of granule proteins (e.g. eosinophil peroxidase (EPX), eosinophil-derived neurotoxin (EDN) and eosinophil cationic protein (ECP) among others) that are highly basic (pI ~ 9–11), cytotoxic and exhibit membrane-destabilizing properties⁶, especially if misfolded. However, the absence of prominent granule proteins is also deleterious for successful eosinophil differentiation as *Mbp1*^{-/-}*Epx*^{-/-} mice with dual deletions of the most abundant eosinophil lineage-specific cationic granule proteins⁷, were also eosinophil deficient. These data suggest that successful eosinophil differentiation requires the temporally and quantitatively appropriate production of different granule proteins as well as their proper folding. A failure of either process causes defective granule formation and cell death.

The mice lacking XBP1 show normal GMP differentiation and survival as well as commitment to EoPs². These findings are somewhat unexpected as *Mbp1*^{-/-}*Epx*^{-/-} mice

showed a failure of GMP proliferation and differentiation commitment to EoPs⁷. All known positive (GATA-1, GATA-2, C/EBPs, PU.1 and Id2) and negative regulators (FOG1 and Id1) for eosinophil development are expressed normally in *Xbp1*-null GMPs². These results indicate that XBP1 does not regulate the expression of MBP1 and EPX by GMPs, although these genes are essential for the GMP to EoP transition. One possibility is that the activation of alternative UPR pathways such as PERK and ATF6 in *Mbp1*^{-/-}*Epx*^{-/-} cells contribute to resolving misfolded-protein-induced ER stress and enable the GMP commitment to EoP. Irrespective of mechanism, the data by Bettigole *et al.* show that eosinophil granulogenesis is a critical developmental checkpoint and if defective, disrupts lineage-specific regulatory events required for continued self-renewal and cell survival. It remains to be determined if cell death is caused by impaired granulogenesis *per se*, cytoplasmic release of toxic proteins⁸, ER collapse⁹ or other processes.

In summary, Bettigole *et al.* provide to the research community a novel eosinophil-deficient mouse strain. These animals will complement the currently available PHIL¹⁰ and dbIGATA mice¹¹, which while eosinophil deficient, were created by lineage-specific diphtheria toxin expression or *Gata1* gene ablation, respectively. This new mouse will enable exploration of the cell type specific relationship between XBP1 and key developmental regulators GATA-1 and GATA-2 and how XBP1 regulates granule protein production and assembly, UPR and ER stress in eosinophil precursors. It is yet to be determined how well these mice will model eosinophilic diseases such as allergic asthma as XBP1 loss affects DC numbers and is expressed by multiple other mature immune cells. Nonetheless, the *Xbp1*-null, PHIL and dbIGATA mice provide an impressive and expanding tool-box to help dissect the essential roles of eosinophils in health and human diseases.

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Figure 1. XBP1 is required for eosinophil differentiation

Deletion of XBP1 in HSCs prevents granulopoiesis in EoPs, inducing apoptosis and a profound eosinophil deficiency². Other immune cells are unaffected in number although their functionality remains to be examined². In mouse EoPs, defined by the expression of IL-5Rα (immature and matured cells) and CCR3 (matured cells), develop from GMPs. HSC, hematopoietic stem cell; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; Baso, basophil; Neut, neutrophil; B, B cell; T, T cell; NK, natural killer cell; Mono, monocyte; EoP, Eosinophil progenitor; GMP, granulocyte-monocyte progenitor; IL-5Rα, IL-5 receptor alpha; CCR3, CC chemokine receptor 3.