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Transcription factor and cytokine regulation of eosinophil lineage commitment

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Abstract

Purpose of review: Lineage commitment is governed by instructive and stochastic signals which drive both active induction of the lineage program as well as repression of alternative fates. Eosinophil lineage commitment is driven by the ordered interaction of transcription factors, supported by cytokine signals. This review summarizes key findings in the study of eosinophil lineage commitment and examines new data investigating the factors that regulate this process.

Recent findings: Recent and past studies highlight how intrinsic and extrinsic signals modulate transcription factor network and lineage decisions. Early action of the transcription factors C/EBP α and GATA-1 along with C/EBP ϵ supports lineage commitment and eosinophil differentiation. This process is regulated and enforced by the pseudokinase Trib1, a regulator of C/EBP α levels. The cytokines IL-5 and IL-33 also support early eosinophil development. However, current studies suggest that these cytokines are not specifically required for lineage commitment.

Summary: Together, recent evidence suggests a model where early transcription factor activity drives expression of key eosinophil genes and cytokine receptors to prime lineage commitment. Understanding the factors and signals that control eosinophil lineage commitment may guide therapeutic development for eosinophil mediated diseases and provide examples for fate choices in other lineages.

Keywords

hematopoiesis; eosinophil lineage commitment; eosinophilopoiesis; transcription factors; cytokines

Introduction

Hematopoietic cells depend on a finely balanced network of signaling pathways and transcription factors to progress from multipotent progenitors to terminal effectors and maintain cellular identity. This requires that cells specify a lineage and exclude all others. These are active processes and require multiple levels of regulation. Cytokines deliver cell extrinsic instructive signals which are balanced by instructive and stochastic cell intrinsic regulation through transcription factor networks. While hematopoiesis is classically depicted

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as discrete, stepwise commitment to progressively more restricted progenitors [1], recent work at the single-cell level demonstrates that early progenitors are pre-committed or restricted to particular lineages [2–5**]. This shift in our understanding of hematopoiesis underscores the need to re-examine the process by which cells commit to specific lineages and maintain those lineage identities to maturity.

Eosinophils are characterized by a highly granular cytoplasm rich in destructive cationic proteins, cytokines, and chemokines, pre-formed for rapid release upon activation [6–11]. Eosinophils were initially described as critical for defense against parasites [12, 13]. However, several recent studies raise questions about the protective role of eosinophils in murine helminth infections [14–16]. Similarly, eosinophils expand under atopic conditions and can have either pathologic [17–19] or protective functions [20, 21]. Together, these reports provide insights into the important roles that eosinophils play at steady state and under stress and highlight the need to understand their origin and identity. In this review, we survey the current understanding of the factors and signals that initiate and support eosinophil lineage commitment and its relationship to our evolving understanding of hematopoiesis.

Cell lineage choice is set early

Recent work at the single-cell level demonstrates that myeloid progenitor populations are highly heterogeneous and that many of these cells are already pre-committed to a specific lineage [2–5**]. A key finding of these studies is that cell type-specific transcription factor networks are either primed or expressed in early progenitors. Of relevance here, these studies identified clusters of cells expressing eosinophil-specific genes, particularly among phenotypic GMPs [4], suggesting that at the single-cell level, lineage potential is restricted much earlier than previously thought. While much has been learned recently regarding when these cells develop a lineage identity, the triggers for these early steps in lineage determination remain unknown.

Myeloid lineage commitment

Although the recent single-cell studies of myelopoiesis are changing the way we think about hematopoiesis, the majority of data regarding the early steps in eosinophil lineage commitment was generated from bulk sorted bone marrow cells or *in vitro* expression systems. Nevertheless, this stepwise approach provides a framework for understanding the early steps in eosinophil commitment. As most of our understanding of this process derives from studies of mouse hematopoiesis, we will focus largely on those data here. However, the majority of these factors are conserved in humans. Murine eosinophils pass through several well-described intermediates as they differentiate from the hematopoietic stem cell (HSC). In particular, the common myeloid progenitor (CMP) and the granulocyte/macrophage progenitor (GMP) were shown through transplant studies to give rise to all granulocytic lineages [22].

Eosinophil progenitors set the transcriptional landscape for mature eosinophils

Eosinophils pass through a lineage-committed progenitor stage, termed the eosinophil progenitor (EoP), which is derived from the GMP in mice [23*] and from the CMP in

humans [24]. This difference between mouse and human may reflect an earlier commitment to the eosinophil lineage in humans, however this needs to be explored at the single-cell level. As cells progress from the GMP to the EoP, they undergo significant transcriptional changes, with the induction or repression of nearly 500 genes including induction of key granule protein genes such as eosinophil peroxidase and major basic protein (*Epx*, *Prg2*) [25*]. Many of the genes repressed at this transition are associated with other myeloid lineages, such as myeloperoxidase (*Mpo*), neutrophil elastase (*Elane*), proteinase 3 (*Prtn3*), and cathphesin G (*Ctsg*), demonstrating that active repression of non-eosinophil lineage genes is also required for lineage commitment. Interestingly, a larger subset of genes, nearly 1200, were altered in expression during the EoP to mature eosinophil transition [25*], suggesting that identity formation continues long past lineage commitment.

Transcription factor networks in eosinophil lineage commitment and development

Multiple transcription factors are required for eosinophil development, including C/EBP α , C/EBP ϵ , IRF8, PU.1, GATA-1 and GATA-2. These factors are differentially required to specify the eosinophil program and fully commit to that lineage and they operate in a network that responds to external signals (Fig. 1). As discussed below, much is known regarding the interactions between these transcription factors, however significant gaps remain in our understanding.

C/EBP α drives myeloid lineage commitment and eosinophil transcriptional networks—The initial steps in eosinophil lineage commitment/specification are driven largely by C/EBP α and GATA-1 and the factors that regulate their levels. C/EBP $\alpha^{-/-}$ mice lack neutrophils and eosinophils, due to a block at the CMP to GMP transition [26, 27*]. In studying the role of C/EBP proteins in eosinophil development, Nerlov, Graf, and colleagues observed in transformed chicken multipotent progenitors that C/EBP α expression induced eosinophil lineage commitment [28]. C/EBP α also induces the expression of other transcription factors that are required for later eosinophil development, including C/EBP ϵ [29**]. In other lineages, cells are sensitive to graded levels of C/EBP α with neutrophils favoring high levels and monocytes requiring low levels of C/EBP α for development [29**]. As we note below, C/EBP α expression in eosinophils must also be precisely tuned.

The balance of GATA factors and FOG-1 modulates eosinophil commitment—Eosinophils require GATA-1 for development, as mice with a mutation in the *Gata1* promoter, lack eosinophils [30]. This mutation decreases *Gata1* levels by ~75% in basophils [31]. Furthermore, eosinophil potential is restricted to GATA-1⁺ myeloid progenitors [2]. Furthermore, eosinophil-lineage primed GMPs were also *Gata1*⁺ at the single cell level [4]. In human hematopoietic progenitors, ectopic-expression of either GATA-1 or GATA-2 alone was sufficient to promote eosinophil differentiation [32]. In contrast, ectopic C/EBP α expression yielded both neutrophils and eosinophils. In the transformed chicken multipotent progenitor system, expression of intermediate levels of GATA-1, in conjunction with C/EBP β , another C/EBP family member, generated eosinophils. In contrast, high levels of GATA-1 alone failed to induce eosinophil differentiation [33**, 34*]. Eosinophil differentiation correlated with decreasing levels of the GATA co-factor, FOG-1. Conversely, forced expression of FOG-1 blocked GATA-1-mediated eosinophil differentiation. This

process was antagonized by PU.1, which down-regulated GATA-1 in multipotent progenitors [35]. Importantly, the antagonistic relationship between FOG-1 and GATA-1 is regulated by C/EBP α .

While cooperative networks of transcription factors can reinforce or oppose cell-type specific gene expression, a study from Iwasaki *et al* illustrated that there are graded differences in the co-expression of C/EBP α and GATA-2. The authors used ectopic expression of C/EBP α and GATA-2 in purified common lymphoid progenitors (CLPs) and temporally regulated their expression [36**]. Their results demonstrated that expressing C/EBP α prior to GATA-2 generated eosinophils. In contrast, expressing GATA-2 prior to C/EBP α generated basophils. This is in line with other work indicating that C/EBP activity is required to down-regulate FOG-1 [33**]. C/EBP β expression induced downregulation of FOG-1, allowing for GATA-1 activity and eosinophil development. Ectopic FOG-1 expression inhibited C/EBP β -mediated transcription as well, suggesting a mutually antagonistic relationship [33**]. As C/EBP α and C/EBP β have partly overlapping functions in eosinophil development [28], this work suggests that C/EBP α primes cells to be responsive to GATA-1 via FOG-1 inhibition.

Early IRF8 expression, which drives *Gata1* transcription, is also required to induce the eosinophil fate. IRF8-deficient mice showed a reduction in EoPs as well as in EoP *Gata1* expression [37]. GATA-1 also interacts with PU.1 and C/EBP ϵ to modulate eosinophil granule protein production [38, 39*]. PU.1 is required at earlier stages for myeloid differentiation [38, 40, 41]. It is unclear if PU.1 expression is truly required for eosinophil lineage commitment however, as PU.1-deficient fetal liver cells retain expression of *Epx* (eosinophil peroxidase) and *Prg2* (major basic protein) [42]. In addition, as noted above, PU.1 repressed GATA-1 expression [35], making its role less clear.

Taken together, these studies indicate that eosinophil lineage commitment requires a decrease in FOG-1, driven by C/EBP α , and increased IFR8 to unleash GATA-1 activity. GATA-1 then acts in concert with C/EBP α to specify and maintain the eosinophil lineage. In the above studies, C/EBP β and GATA-2 were able to compensate for C/EBP α and GATA-1, respectively, however it is unclear if this occurs *in vivo*. The precise mechanism of the C/EBP α -GATA-1 relationship is unknown and awaits a detailed analysis of transcription factor binding during eosinophil development, which has been hampered by the ability to obtain sufficient numbers of these rare eosinophil progenitors.

C/EBP ϵ functions both early and late in eosinophil maturation—Lineage commitment also requires the active maintenance of a lineage program. The process of granule production is critical to eosinophil development. C/EBP ϵ is required for granule protein expression and terminal granule maturation in both eosinophils and neutrophils [39, 43]. C/EBP $\epsilon^{-/-}$ mice lack eosinophils and present with atypical and defective peripheral neutrophils as well as myelodysplasia [44]. In humans, C/EBP ϵ has 4 different isoforms with differential abilities to promote development [45]. In addition, the larger isoforms, $\epsilon 32$ and $\epsilon 30$ are transcriptional activators, whereas the smaller isoforms, $\epsilon 27$ and $\epsilon 14$, are transcriptional repressors. Expression of the 2 larger C/EBP ϵ isoforms in CD34 $^{+}$ human hematopoietic progenitors drove eosinophil production, independent of IL-5 [46], suggesting

that C/EBP ϵ can support lineage commitment itself. In contrast, the smaller two C/EBP ϵ isoforms strongly inhibited eosinophilopoiesis, even in the presence of IL-5. Of note, mice only possess two C/EBP ϵ isoforms that are reported to have similar functions[47].

These data suggest a model where developing progenitors upregulate expression of C/EBP α and IRF8 while downregulating FOG-1. This allows for an increase in the expression of both GATA-1 and C/EBP ϵ , turning on eosinophil-specific gene expression and suppressing alternative lineage programs. Critical to this process is the regulation of these transcription factors and below, we discuss a key regulator of C/EBP α protein levels.

Trib1 controls eosinophil lineage commitment and terminal identity

Recent studies implicate the Tribbles pseudokinase family in eosinophil lineage commitment and development. Tribbles proteins function primarily as adaptors to promote protein degradation and/or sequestration [48–50]. There are three mammalian tribbles homologues (Trib1–3), defined by a central serine/threonine kinase-like domains and C-terminal sequences that bind the E3 ubiquitin ligase COP1 [51–54]. Mice with a germline deletion of Trib1 lack eosinophils and “M2”-polarized macrophages and have more neutrophils [55*]. This phenotype is influenced by the failure of Trib1 to promote C/EBP α protein degradation [55*]. Myelopoiesis is unaffected in mice lacking Trib2 or Trib3 [55, 56]. Trib1 selectively interacts with C/EBP α p42 and not C/EBP α p30 [57]. As a consequence, ectopic Trib1 expression in mouse bone marrow progenitors lead to a p30-driven leukemia due to the loss of p42 antiproliferative and pro-differentiation effects [54].

While previous work revealed alterations in myeloid populations with Trib1 loss, the precise mechanisms were not well understood. In addition, it was unknown when and how Trib1-mediated regulation of C/EBP α protein expression [55*] impacted eosinophil development. It was also unclear if eosinophils required a low or high level of C/EBP α for their development. Our group found that Trib1 regulates eosinophil precursor lineage commitment from the GMP as conditional Trib1 deletion in HSCs reduced the size of the EoP pool [58*]. We further demonstrated that Trib1 suppressed the neutrophil program in lineage-committed eosinophil precursors in response to IL-5. Our study demonstrated that Trib1 normally reduces eosinophil C/EBP α p42 protein expression. In the absence of Trib1, C/EBP α p42 increased, which was associated with both more neutrophils and eosinophils with neutrophil characteristics, including surface Ly6G expression, increased phagocytic capacity, and the presence of both eosinophil- and neutrophil-type cytoplasmic granules [58*]. Some of these features are also seen in activated eosinophils [59–61], suggesting a functional impact of Trib1 in these cells. Loss of Trib1 caused eosinophil retention in the bone marrow, likely due to an increase in eosinophil CXCR4 expression. Our data demonstrate that eosinophils require lower levels of C/EBP α compared to neutrophils, as increased C/EBP α caused the cells to develop a more neutrophilic identity. While C/EBP α is thought to modulate the majority of this phenotype, Trib proteins are known to interact with other regulators of cell function including AKT and MAPK [62–65], raising the possibility of alternative mechanisms. Together, these findings provide new insights into early steps in eosinophil development, where Trib1 controls eosinophil lineage commitment

from the GMP and suppresses C/EBP α and the neutrophil program in response to IL-5. These combined actions promote eosinophil lineage commitment and fidelity.

Cytokine signals support eosinophil lineage development

An additional function of the transcription factor networks described above is to facilitate responsiveness to extrinsic cytokine signals. In particular, the cytokines IL-5 and IL-33 are critical for eosinophil development and will be discussed below.

IL-5 signaling enforces eosinophil development—The EoP expresses the high affinity alpha chain of the IL-5 receptor (IL-5R α) [23] and IL-5 drives eosinophil development and proliferation *in vivo* [66*] and *ex vivo* [67, 68]. Furthermore, eosinophilia is observed in IL-5 transgenic mice whose T cells constitutively express IL-5 [69]. Despite these data, IL-5-deficient mice retain homeostatic levels of eosinophils [70*], suggesting that other factors support steady-state eosinophil production. Furthermore, it is unclear what drives IL-5R α expression and if its expression is a consequence or a cause of eosinophil lineage commitment.

Recent work demonstrated that IL-5 promotes a network of factors that shape eosinophil development. IL-5 stimulation induced IL-4/IL-4R α expression in developing eosinophils and this interaction supported eosinophil expansion [71]. In the absence of IL-5, IL-4 inhibited eosinophil survival *ex vivo*. In contrast, CCL3 produced by immature eosinophils acted in an autocrine fashion to stimulate eosinophil maturation in the absence of IL-5. Together, these data suggest that IL-5, while not required for the initial steps in eosinophil lineage commitment, enforces eosinophil lineage programming by increasing eosinophil responsiveness to other growth signals and supporting eosinophil-specific gene expression.

IL-33 supports eosinophilopoiesis—The cytokine IL-33 is also implicated in eosinophil lineage commitment and development. IL-33 is an alarmin IL-1 family member released following epithelial damage [72] and activates Th2 cells and ILC2s to produce IL-5 and IL-13 [73–76]. It subsequently drives eosinophilia when administered to mice [77]. Eosinophils express the IL-33 receptor (ST2) and upregulate it upon recruitment to the airway after allergen challenge [78]. IL-33 activates eosinophils [79] and promotes cell survival in conjunction with GM-CSF [80].

These reports highlight the role of IL-33 in the development of eosinophilia following challenge, yet these functions are largely dependent on IL-33-induced IL-5. Studies attempting to delineate an IL-5-independent role for IL-33 in eosinophils development struggled to separate the two and there are conflicting reports on the ability of IL-33 to promote eosinophil development *ex vivo*. One study reported that IL-33 promoted eosinophil production from c-Kit⁺ BM progenitors *ex vivo* [78]. In contrast, another report found that IL-33 did not support eosinophil production *ex vivo* and, in fact, antagonized IL-5-driven eosinophil production [81]. Finally, IL-33- or ST2-deficient mice show reduced steady-state eosinophil levels [82]. While IL-33 required IL-5 to promote eosinophil production *in vivo*, the inverse was also partly true. IL-5 transgenic mice lacking ST2 had a partial decrease in eosinophilia [82]. ST2 knockout mice also showed reduced neutrophils, suggesting that IL-33 plays a role in neutrophil development.

Taken together, IL-33 and IL-5 are interconnected and interdependent in their roles in eosinophil development, and more work is required to delineate separate roles for IL-33 in eosinophilopoiesis. Furthermore, while lineage commitment can occur in the absence of IL-5 and IL-33, the signals that drive eosinophil lineage commitment remain unknown.

Conclusion

Eosinophils serve many functions both at homeostasis and under settings of infection or inflammation. A large body of work investigated both the transcription factors and cytokine signals that support eosinophil lineage commitment and development. From this, a model emerges where GATA-1 and C/EBP α drive the early steps in lineage commitment, and a network of cytokines, including IL-5 and IL-33, acts in concert with C/EBP ϵ to enforce eosinophil-specific gene expression and support later steps in maturation. Finally, the pseudokinase Trib1 is a key regulator of eosinophil commitment and identity. By precisely tuning lower levels of C/EBP α in EoPs and in developing eosinophils, Trib1 represses alternative fates and enforces eosinophil lineage programming, maintaining eosinophil identity. While much has been learned recently, especially using single-cell analysis, much remains to be understood, particularly the specific signals for eosinophil lineage commitment itself. Further high-resolution studies will provide a path forward to understanding how cells determine lineage specificity, exclude alternative fates, and maintain their identity into maturity.

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Key points

- Eosinophil lineage commitment occurs early in hematopoiesis, likely in the CMP or GMP and is mediated by the ordered expression of C/EBP α and GATA-1, supported by IRF8 and decreasing FOG-1 expression.
- Trib1, a pseudokinase, modulates the levels of C/EBP α following differentiation from the GMP to enforce the eosinophil fate and repress the neutrophil program in developing eosinophils.
- The cytokines IL-5 and IL-33 work together to support eosinophil lineage commitment and development under normal and stress settings, however, they are not specifically required for lineage commitment itself.

Figure 1.

Model for eosinophil lineage commitment. Early transcription factor activity drives lineage commitment, granulogenesis and maturation as well as priming cells to be responsive to cytokine signals.