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2025

Original citation:
Gatti, D. M., & Reynolds, L. A. (2025). Thymic eosinophils: What are you doing here? *Journal of Leukocyte Biology*, *117*(4). https://doi.org/10.1093/jleuko/qiaf001

OXFORD JLB JOURNAL OF LEUKOCYTE BIOLOGY

Journal of Leukocyte Biology, 2025, **117**, qiaf001

https://doi.org/10.1093/jleuko/qiaf001
Advance access publication 8 January 2025
Review

Thymic eosinophils: What are you doing here?

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Abstract

The thymus is a primary lymphoid organ where major types of T lymphocytes undergo essential developmental processes. Eosinophils are among the cell types present in microenvironments within the thymus, and perhaps surprisingly, the role of thymic eosinophils, especially during homeostatic conditions, remains unclear. Major physiological events impact thymic organization and function throughout life, including age-related involution, pregnancy, and exposure to chemotherapy or radiation. In this review, we summarize literature that has explored factors that regulate the accumulation, phenotype, and location of thymic eosinophils during homeostatic development and during conditions in which homeostasis is perturbed. Further, we discuss the current theories as to the function of thymic eosinophils and consider how the heterogeneity of thymic eosinophil populations may reflect a temporal, spatial, and situational multifunctionality of thymic eosinophils.

Keywords: aging, development, eosinophils, homeostasis, thymic damage, thymocytes, thymus

Key Concepts

- Eosinophils are present in the thymus throughout life in humans, mice, and other animals.
- The abundance and characteristics of thymic eosinophils change throughout early life development and into adulthood.
- Thymic eosinophil populations are heterogeneous, in terms of phenotype and spatial location within the thymus.
- The role(s) of thymic eosinophils are beginning to be uncovered, with evidence supporting both roles in thymocyte development and in maintenance or repair of thymic tissue architecture.

Open Questions

- What is the role of thymic eosinophils during major physiological events that can impact thymic cellularity and function such as bacterial or viral infections, physical stress, cancer, or pregnancy?
- Which thymocytes could eosinophils be interacting with, and what is the fate of those thymocytes?
- What role do thymic eosinophils play during aging-related involution, and are these eosinophils functionally different than those that could be interacting with thymocytes?
- Do all eosinophils in the thymus undergo local maturation processes, and if so, what signals stimulate this?
- Do all thymic eosinophils traffic directly to the thymus from the bone marrow?

1. Introduction

The thymus is a primary lymphoid organ responsible for guiding T cell progenitors through selection processes, resulting in mature T cells that will respond to antigen stimulation, undergo effector differentiation, and facilitate host immunity against foreign pathogens and cancers, while minimizing the output of self-reactive T cells. The majority of these developing T cells (thymocytes) will mature into conventional αβ T cells, and these are the thymocytes we will focus on throughout this review. Thymocytes will enter at the vascular corticomedullary junction and migrate through 2 regions of the thymus, the cortex and medulla, to interact with a diverse range of stromal cells (fibroblasts, epithelial cells) and immune cells (including dendritic cells, B cells). These complex interactions between thymocytes and thymic-resident cells are imperative for central tolerance induction by ensuring that (1) thymocytes synthesize a unique T cell receptor (TCR) capable of binding major histocompatibility complex (MHC) class I or class II proteins and (2) thymocytes whose TCR recognizes self-antigen peptides are deleted or acquire a regulatory fate. Processes of selection are and must be rigorous to stop dysfunctional and self-reactive T cells from leaving the thymus and populating the periphery.

T cell production and mounting effective host immunity is predicated on proper functioning within the distinct microenvironments of the thymus. A perhaps unexpected member of multiple thymic microenvironments is the immune cell type, the eosinophil. Eosinophils have been well characterized for their roles in the inflammatory response in type 2 immune contexts. However, recently eosinophils have also received attention for the important, beneficial roles that they play during homeostasis.[1,2] For example, at steady state, eosinophils are involved in (1) tissue morphogenesis of the lungs[3] and mammary glands,[4] (2) protection and repair of the heart[5] and liver,[6] (3) epithelial barrier maintenance in the gastrointestinal tract[7] and lungs,[8] and (4) controlling adipose tissue homeostasis by aiding in glucose metabolism.[9] In the thymus, the functional capabilities of eosinophils have been debated, and there are 2 prevailing hypotheses as to the functions of eosinophils at this site. The first is the potential contributions of thymic eosinophils to thymocyte development, as thymic eosinophils can express proteins required for facilitating thymocyte selection.[10–13] The second involves the maintenance of thymic architecture and tissue structure, as there

Received: October 7, 2024. **Revised:** November 29, 2024. **Accepted:** January 6, 2025. **Corrected and Typeset:** April 25, 2025

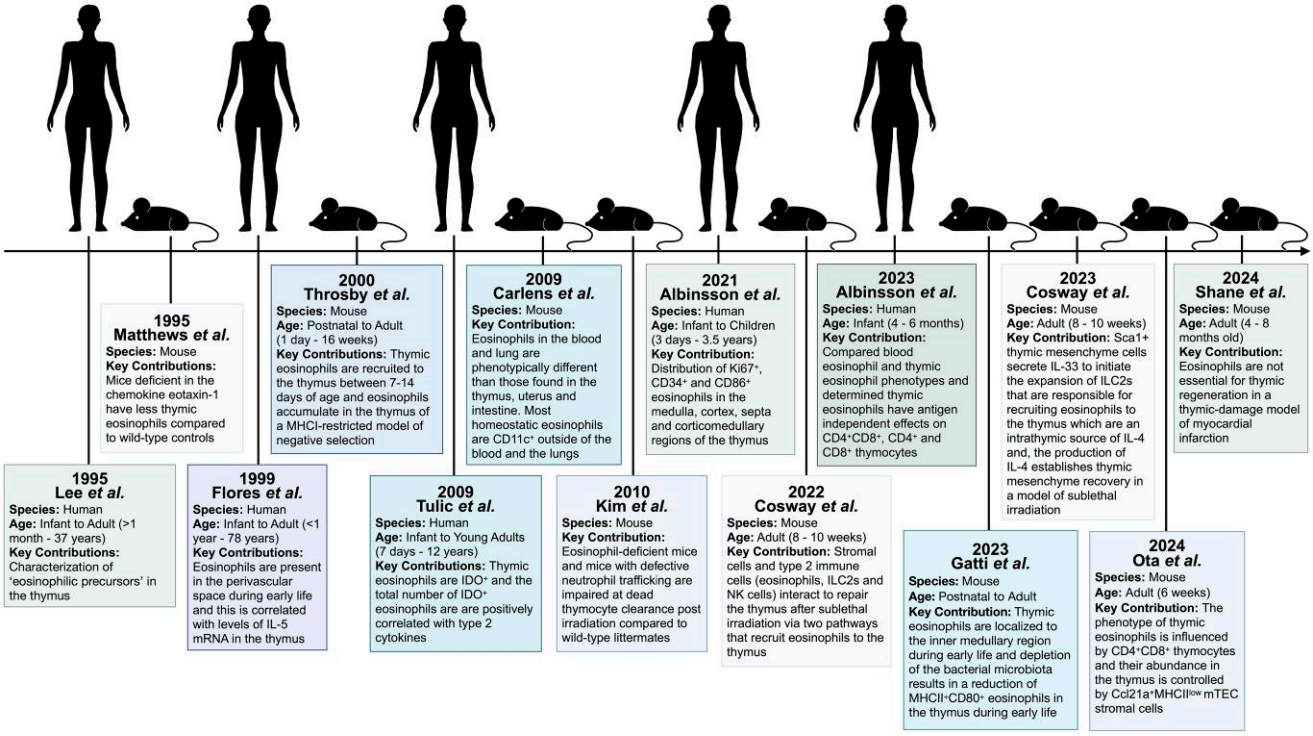

Fig. 1. Key milestones in thymic eosinophil research. Select studies that provided new insights into the regulation and function of thymic eosinophils are highlighted. The species each study focused on, the age (if stated) of subjects being examined, and a summary of each study's key contribution is provided.

is evidence that eosinophils facilitate thymic regeneration after tissue damage and partake in the "clean-up crew" that removes dead or anergic thymocytes.[14,15] The occurrence of these roles could be simultaneous, temporally regulated, or divided among thymic eosinophil subsets. In this review, we visit the literature surrounding the phenotypes, morphologies, regulators, origin, and location of thymic eosinophils and discuss the evidence for the different potential functional roles of thymic eosinophils throughout life.

2. Identifying thymic eosinophils

Throughout the years, thymic eosinophils have been reported in many different animals such as pigs,[16] rabbits,[17] birds,[18] frogs,[19] and guinea pigs,[20] but most recent studies have focused on the characterization and functional roles of thymic eosinophils in mice[12,13,21–23] and humans (Fig. 1).[10,11,24,25] Different techniques and technologies have been used to identify the presence of eosinophils in the thymus. Initially, researchers relied on the distinct cellular morphology of eosinophils (i.e. nucleus shape, numerous cytoplasmic granules, and striking appearance after eosin staining) to identify eosinophils present in thymic histological samples. In 1905, Goodall[20] documented observing "polymorphonuclear leucocytes" with deep eosin staining invading the thymus in newborn guinea pigs. Badertscher in 1920[16] documented eosinophils in the thymus as a cell type with "granules that are coarse, round, [and] quite uniform in size" in histological samples from pigs.

In 1977, biochemical assays allowed Müller to identify thymic eosinophils in humans as histochemically positive for peroxidase, an enzyme that catalyzes the oxidation of a substrate by hydrogen peroxide.[26] Eosinophil peroxidase proteins are a major constituent of cytoplasmic granules, and when eosinophils become activated, eosinophil peroxidase can be secreted into surrounding tissues. Flow cytometry studies allow for identification of thymic eosinophils using side-scatter characteristics and surface protein expression. However, even with complex technology such as flow cytometry, the criteria to identify a thymic eosinophil are still being revised.

In 2000, Throsby et al.[12] characterized thymic eosinophils in mice using the surface markers CD11b and CD11c for identification, which excluded a subset of CD11c$^-$ tissue-resident thymic eosinophils.[13] Currently, protein homologs Siglec-8 and Siglec-F in humans and mice, respectively, are key surface markers for eosinophil identification.[27–29] While Siglec-F expression can be detected on a limited number of other cell types[30] such as alveolar macrophages[31] and intestinal epithelial M cells,[32] there is currently no report of Siglec-F expression on any other thymic-resident cell besides eosinophils. Most recently, single-cell RNA sequencing analysis has provided valuable information about the gene expression profile of thymic eosinophils compared with eosinophils from other tissues.[22]

Specific and comprehensive characterization of thymic eosinophils is challenging because eosinophils are (1) difficult to isolate due to their rarity in the thymus (<0.2% of all thymic cells in mice at steady state)[13,21] and (2) heterogeneous, whereby subpopulations express different surface proteins that change throughout development.[12,13,24] It is likely that thymic eosinophils are divided into phenotypically and morphologically distinct subsets that are playing different roles in the thymus, and perhaps different roles throughout life.

3. Thymic eosinophil phenotypes

A common core repertoire of surface and intracellular proteins has been reported among thymic eosinophils, but substantial

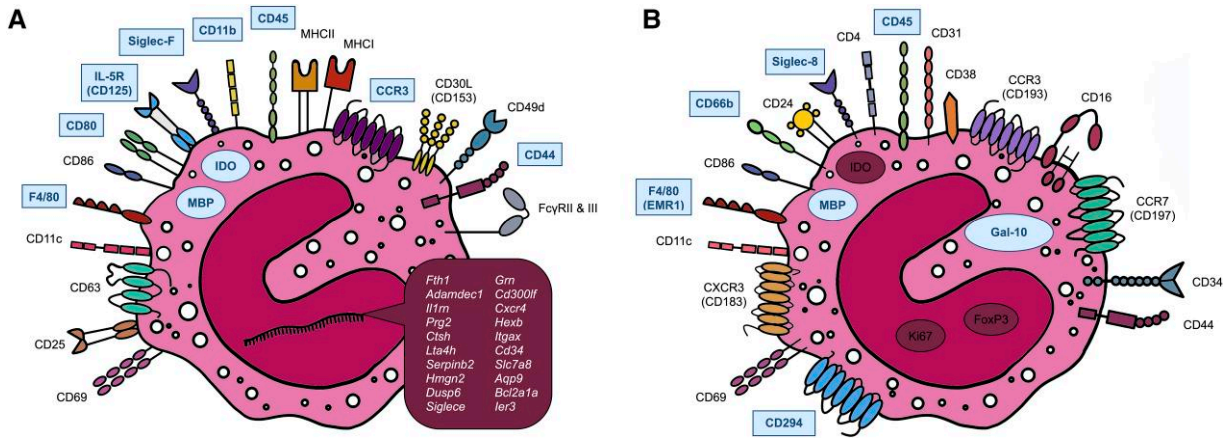

Fig. 2. Phenotypes of mouse and human thymic eosinophils. (A) Intracellular and surface proteins that can be expressed in murine thymic eosinophils are depicted, in which protein names in a blue box or blue circle are expressed by the majority of thymic eosinophils, such as CD45,[12,13,21–23,33] Siglec-F,[13,21–23,33] CD11b,[12,13,21–23,33] F4/80,[12] CD125,[13] CD80,[13] CD44,[12] CCR3,[33] IDO,[13] and major basic protein (MBP),[34] and protein names in a black font, such as MHC class I,[12] MHC class II,[12,13] CD86,[12] CD11c,[12,13,22] CD63,[13] CD25,[12] CD69,[12] CD153,[12] CD49d,[12] FcγII,[12] and FcγIII,[12] have been reported to be expressed by some thymic eosinophils. In the red text box, the top 20 genes that are upregulated in thymic eosinophils relative to eosinophils in the spleen and blood of mice are listed.[22] (B) Intracellular and surface proteins that can be expressed in human thymic eosinophils in which protein names in a blue box or blue circle are expressed by the majority of thymic eosinophils, such as CD45,[10,11] Siglec-8,[10,11] Gal-10,[10,11] CD66b,[10] F4/80,[11] CD294,[10] and MPB,[11] and protein names in a black font, such as CD4, CD24, CD11c, CD183, CD69, CD31, CD38, CD193, CD16, CD197, CD44, FoxP3[10]; CD86[11]; CD34[11]; IDO[24]; and Ki67,[11] have been reported to be expressed by subsets of thymic eosinophils.

heterogeneity in protein expression can occur within thymic eosinophil populations in both mice (Fig. 2A) and humans (Fig. 2B).[10–13,33]

3.1 Mouse thymic eosinophil phenotypes

A notable fraction of murine thymic eosinophils has been reported to express MHC class II along with the costimulatory molecule CD80,[12,13] and interestingly, the frequency of thymic eosinophils that express MHC class II increases in abundance throughout postnatal development (between 1 and 6 wk of age).[13] In another study characterizing mice at 2 wk of age, thymic eosinophils were reported to express low levels of MHC class I and costimulatory molecules CD30L (CD153) and CD86.[12] The implications for expressing MHC class I and II molecules in addition to costimulatory molecules CD80, CD86, and CD30L are that thymic eosinophils would be capable of presenting antigens to developing thymocytes pre and post bifurcation into CD4 and CD8 single positive (SP) thymocytes.

The expression of the membrane-bound protein CD63 has been reported on some murine thymic eosinophils, and there is a steady decrease in the number of thymic eosinophils that express CD63 between 1 and 6 wk of life.[13] The tetraspanin protein CD63 has been implicated in protein trafficking within the hematopoetic lineage and is found on the cell surface and on cytoplasmic secretory granules in immune cells.[35] CD63 has been previously associated with (1) degranulation of mast cells during acute IgE-mediated anaphylaxis,[36,37] (2) extracellular vesicle release concomitant with expelling phagocytosed bacteria in dendritic cells,[38] (3) targeted secretion of elastase in neutrophils,[39] and (4) different types of degranulation in human eosinophils such as piecemeal degranulation and compound exocytosis.[40,41] While the functional implications of cell-surface CD63 expression by thymic eosinophils are not yet known, early reports by Badertscher[16] claim to find "free granules" lying outside of thymic eosinophils in histological samples. What is unknown is the content of these granules; therefore, the possibility remains that eosinophils could be secreting low/limited amounts of products that could be aiding in thymocyte degradation or perhaps tissue remodeling of the thymus. Moreover, we have reported an increase in eosinophil side-scatter characteristic values (indicative of internal cellular complexity) from early life to adulthood, and this inversely correlates with CD63 expression, such that greater internal complexity is associated with lower levels of CD63 expression, possibly indicating less CD63-mediated exocytosis as mice age.[13] Finally, single-cell RNA sequencing analysis revealed that thymic eosinophils specifically express genes associated with degranulation when compared with eosinophils found in the lung or spleen.[22] Altogether, these studies support the possibility of eosinophils undergoing some type of degranulation in the thymus.

CD11c (integrin alpha X chain protein) is a surface marker widely used for defining monocytes, dendritic cells, and macrophages. Carlens et al.[33] showed that eosinophils in the lungs and blood lack CD11c expression; however, most eosinophils in the intestinal lamina propria, thymus, and uterus are CD11c$^+$. Carlens et al.[33] speculated that the small portion of CD11c$^-$ eosinophils in the intestine, thymus, and uterus were eosinophils that had recently immigrated from the blood, suggesting that CD11c was a marker associated with recent tissue entry of homeostatic eosinophils. In line with the CD11c observations made by Carlens et al.[33], Ota et al.[22] showed that in mice CD11c was present on thymic eosinophils, absent on lung eosinophils, and also absent on spleen eosinophils (the latter was not investigated by Carlens et al.). Further, the authors reported that in *Rag1*$^{-/-}$ and *Rag2*$^{-/-}$ mice, in which thymocyte differentiation is arrested at the double negative (CD4$^-$CD8$^-$) stage of development, the number of thymic eosinophils expressing CD11c significantly decreased, whereas in *Tcra*$^{-/-}$ mice, in which thymocyte differentiation is arrested at the double positive (DP) (CD4$^+$CD8$^+$) stage of differentiation, there was no change in the eosinophil phenotype.[22] These findings suggest that DP thymocytes (absent in *Rag1*$^{-/-}$ and *Rag2*$^{-/-}$ mice) may play a role in establishing thymic eosinophil surface protein expression.[22] Altogether, it can be speculated that recently immigrated thymic eosinophils from the blood upregulate CD11c expression once inside the thymus after receiving signals from DP thymocytes.

3.2 Human thymic eosinophil phenotypes

Albinsson et al.[10] utilized thymic tissue removed from patients undergoing a corrective heart surgery to compare the surface

marker expression of blood eosinophils to thymic eosinophils in infant patients. Blood eosinophils and thymic eosinophils were reported to be phenotypically different from one another, and while blood eosinophils exhibited interindividual differences across patients, thymic eosinophils, although heterogeneous, varied little between patients.[10] This supports the idea that "thymic eosinophils" become a distinct entity once encountering intrathymic tissue cues.[10] In all patients, a significantly larger portion of thymic eosinophils were found to express CD31 (PECAM-1) relative to eosinophils collected from the blood.[10] In the thymus, it has been shown that $CD31^+$-transfected thymic stromal cells can use CD31 to induce apoptosis of DP thymocytes.[42] Therefore, it was speculated that the upregulation of $CD31^+$ on thymic eosinophils in the presence of DP thymocytes could suggest a possible role for eosinophils in modulating apoptosis in developing T cells.[10]

IDO is an intracellular enzyme capable of catabolizing the amino acid tryptophan into immunosuppressive products known as kynurenines.[43,44] It has been shown in vitro that eosinophils can polarize T cell populations using IDO to produce kynurenines that induce apoptosis and inhibit cell proliferation of T helper 1 but not T helper 2 cells.[45] Eosinophils in the thymus of both humans and mice have been shown to be IDO^+ in early life.[13,24] Tulic et al.[24] speculated that eosinophils could be using IDO to skew early-life development of the immune system to favor T helper 2 immunity; however, it is not currently thought that the T helper fate of a naïve T cell is primed in the thymus. Alternatively, IDO is present in signaling cascades of many different immune cell types such as plasmacytoid dendritic cells,[46] cytotoxic T cells,[47] and macrophages.[48] Therefore, it could be that IDO is aiding in an unknown mechanism outside of immunomodulation, but more studies are required to identify the role of IDO in thymic eosinophils.

4. Origin and maturation of thymic eosinophils

Several research groups have tackled the question: where do thymic eosinophils originate from? Researchers in the early 1900s debated the hematopoietic capabilities of the thymus and it was initially thought that tissue-resident stem cells within the thymus could be producing thymic eosinophils.[16,20] While it is now accepted that instead, eosinophils originate from stem cells in the bone marrow, it is still possible that eosinophils exiting the bone marrow could undergo maturation processes after arriving in the thymus.

Lee et al.[25] hypothesized that bone marrow–derived eosinophilic precursors undergo terminal differentiation within the thymus, and the authors searched for evidence of thymic eosinophils progression from a precursor state ("large, premature nuclei which were round, ovoid or band-form" and, "[their] nuclear/cytoplasmic ratio was high and the chromatin was diffusely dispersed") to a mature state. The eosinophilic precursors were found to be most abundant in early life (1 month to 1 year of age) and virtually absent in adulthood (18 to 37 yr of age),[25] indicating that thymic eosinophils arising from stem cell eosinophilic precursors is a process specific to early life thymic development. Similarly, Albinsson et al.[11] documented the presence of eosinophils that resembled different stages of development from eosinophilic precursors (large round nucleus) to mature eosinophils (bilobed nucleus) in the human thymus and identified a subset of thymic eosinophils expressing CD34 (an eosinophilic precursor marker) predominantly found in the septa of the thymus. Additionally, in adult mice Ota et al.[22] investigated differentially expressed genes between thymic eosinophils compared with lung and spleen eosinophils and found that *Cd34* was among the top 20 genes enriched specifically in thymic eosinophils. Altogether, these findings support the idea that maturation of thymic eosinophils could occur locally in the thymus from eosinophil precursors, perhaps as a result of receiving tissue-specific cues from the thymic environment.

5. Signals regulating thymic eosinophil abundance

The signals required for eosinophil entry into the thymus during homeostatic development are beginning to be elucidated. Eotaxin chemokines have been shown to dictate eosinophil migration into tissues in both humans and mice. In humans, there are 3 eotaxin chemokines (eotaxin-1 [CCL11], eotaxin-2 [CCL24], and eotaxin-3 [CCL26]), and there are 2 eotaxin chemokines in mice, eotaxin-1 (CCL11) and eotaxin-2 (CCL24).[49] Thymic eosinophils in mice and humans have been shown to express the transmembrane protein CCR3 (CD193), which interacts with all 3 eotaxins.[33] Matthews et al.[34] reported that eotaxin-1 RNA was detectable in the thymus of mice, and eotaxin-1-deficient mouse models have reduced levels of thymic eosinophils compared with wild-type control mice, indicating that eotaxin-1 could be one of the regulators for baseline thymic eosinophil levels in mice. Consistent with the findings that eotaxins recruit eosinophils to the thymus, Cosway et al.[21] showed eosinophils to be virtually absent in the thymi of *Ccr3*$^{-/-}$ mice (mice deficient in the eotaxin receptor), and in a model of sublethal irradiation, a transfer of eosinophils treated with a CCR3 antagonist prevented the accumulation of eosinophils in the thymus relative to mice given a transfer of eosinophils alone. Further work by Cosway et al. showed that the source of this eotaxin-1 was a subset of medullary thymic epithelial cells (mTECs) with low MHC class II and CD80 expression levels.[21] In another independent study by Ota et al.,[22] the authors showed that the number of eosinophils and the number of mTECs were positively correlated in the thymus. RNA sequencing analysis revealed that a population of $Ccl21a^+$ $MHCII^{low}$ mTECs highly expressed eotaxin-1, which may be responsible for eosinophil recruitment at steady state.[22]

Interleukin (IL)-5 is a cytokine that has been shown to elicit eosinophil migration into tissues such as the gut, uterus, and adipose tissue[50–52] During homeostatic conditions, detectable levels of IL-5 have been reported in the thymus,[13,24] and we have reported that thymic eosinophils maintain expression of CD125 (the IL-5 receptor) throughout life.[13] Throsby et al.[12] showed in mice that thymic eosinophils do not contain IL-5 messenger RNA; therefore, it is unlikely that eosinophils are the source of intrathymic IL-5. Type 2 innate lymphoid cell(ILC2)s are an important cell type for IL-5 secretion in tissues such as the lungs,[53] and work by Cosway et al.[21] showed that ILC2s in the thymus produce IL-5. Therefore, it is likely that ILC2s mediate eosinophil recruitment to the thymus via IL-5 secretion because both IL-5–deficient mice and ILC2-deficient mice have significantly reduced numbers of thymic eosinophils.[21] Additionally, Cosway et al. showed that in a model of sublethal irradiation, ILC2-deficient mice were unable to recover thymic cellularity as effectively as wild-type control mice, and this phenotype was rescued through the administration of recombinant IL-5, which recovered thymic eosinophil levels and thymic tissue cellularity.[21] Altogether, these data suggest that at steady state, ILC2s are important for supporting eosinophils populations in the thymus, and in a model of thymic

damage, IL-5 can promote thymic eosinophil accumulation and thymic regeneration.

The microbiota have a bidirectional relationship with tissue-resident eosinophils in the gut and lungs,[54,55] and we have previously reported that the bacterial gut microbiota play a role in regulating eosinophils in the thymus.[13] Using an antibiotic-depletion strategy in pregnant dams, we showed that depletion of the bacterial gut microbiota led to a significant reduction in the total number and frequency of thymic eosinophils among leukocytes in neonatal mouse pups.[13] We observed that the thymic eosinophils of antibiotic-exposed pups showed a significant reduction in MHC class II expression and granularity indicating that these eosinophils were phenotypically different than those present in non–antibiotic-exposed control pups.[13]

6. Thymic eosinophil location

The thymus comprises 2 lobes connected at the center, and each lobe is surrounded by a thick capsule from which connective tissue extensions called septae/trabeculae divide interthymic lobules. Underneath the capsule, the thymus is divided into an outer cortical region and an inner medullary region where functionally different thymic epithelial cells reside, forming microenvironments conducive to different thymocyte maturation processes. A less defined region between these two compartments is the corticomedullary junction, which is often referred to as the perivascular space, as it is highly vascularized and the entry point of most T cell progenitors. There have been many studies documenting the locations of eosinophils within the thymus, and these locations change depending on the species examined and the age of the study subject.

6.1 Location of mouse thymic eosinophils

Throsby et al.[12] showed that eosinophils in mice are clustered around the corticomedullary junctions during early life (2 to 3 wk of age) but are distributed evenly in the medullary region later in life (16 wk of age). In line with their observations, we have reported a higher frequency of eosinophils in the interior regions of the thymus.[13] Interestingly, the frequency of MHC class II expression in eosinophils appears to increase at the innermost regions of the thymus, indicating that those in the inner medullary regions may be functionally different than thymic eosinophils present in outer cortical regions.[13]

6.2 Location of human thymic eosinophils

Studies using thymic samples from infants to children (3 d to 10 yr) have described eosinophils to be predominantly localized to the corticomedullary junction and medullary region and less numerous in the cortical region and septa/trabeculi of the thymus.[11,24,26,56] Within the medullary region, there are structures called Hassall's corpuscles, where eosinophils have been reported to cluster within and around in the early perinatal period to 4 wk after birth.[15,24,26] Hassall's corpuscles are comprised of keratinizing epithelial cells, and despite their prevalence within the thymus, it remains unclear what their role is. One of the early theories, from studies looking at guinea pigs, hypothesized that Hassall's corpuscles remove apoptotic thymocytes because they appear to be full of cellular debris and phagocytic macrophages.[57,58] However, more recent reports show that epithelial cells within Hassall's corpuscles express thymic stromal lymphopoietin, which has been shown to activate CD11c$^+$ dendritic cells to upregulate costimulatory markers CD80 and CD86, both of which are critical in thymocyte interactions.[59] Eosinophils have also been shown to express CD80 and CD86 in the thymus[12,13]; therefore, eosinophils located in and around Hassall's corpuscles could be upregulating markers critical for interacting with thymocytes in a similar fashion to dendritic cells. Moreover, Tulic et al.[24] reported that the clustering of eosinophils around Hassall's corpuscles is a phenotype more often seen in young children and rarely observed in older children.

It is unknown what controls the altering patterns of eosinophil distribution within the thymus throughout life. Perhaps aging influences the rate of thymic entry to the thymus from the blood, resulting in more eosinophils being detected closer to the point of entry—the corticomedullary junction—in younger animals. It is also possible that differing signals from thymic epithelial cells, thymocyte subsets, or other thymic-resident cells throughout the aging process influence eosinophil trafficking and distribution. These age-related changes in thymic eosinophil distribution could be indicative of changes in the functional roles of eosinophils throughout life.

7. Functional roles of thymic eosinophils

There are 2 major theories regarding the functional role of thymic eosinophils: (1) eosinophils are contributing to the processes of thymocyte development and (2) eosinophils are aiding in thymic tissue maintenance (i.e. repairing thymic damage and helping with disposal of apoptotic thymocytes). Because thymic eosinophils are quite heterogeneous, it is likely that different subsets of thymic eosinophils are fulfilling different roles during distinct periods of life.

7.1 Eosinophils in thymocyte education

Eosinophils have the capability to act as antigen presenting cells and express costimulatory molecules under activating conditions such as during airway inflammation and in the presence of parasitic worms.[60–63] That a sizeable population of thymic eosinophils express MHC classes I[12] and II,[13] CD80,[13] and CD86[12] during postnatal development in mice suggests that thymic eosinophils could have a role in antigen presentation and thymocyte selection. Moreover, Ota et al.[22] revealed that in mice thymic eosinophils exhibited significant upregulation of genes associated with regulating both αβ T cell activation and γδ T cell differentiation when compared with eosinophils present in the lung and spleen. Throsby et al.[12] used multiple models of MHC class I– and II–restricted selection in 2-wk-old TCR transgenic mice to examine the effect of acute selection on thymic eosinophil populations. After administration of the TCR's cognate peptide in a model of class I–restricted selection, eosinophils were recruited to the thymus and appeared to cluster around apoptotic bodies.[12] However, in a model of MHC class II–restricted selection, administration of a cognate peptide had no effect on eosinophil recruitment despite a significant increase of apoptotic thymocytes accumulating in the thymus.[12] This study suggests a preference of thymic eosinophils to accumulate in the thymus during MHC class I–mediated selection of SP CD8$^+$ thymocytes over MHC class II–mediated selection of SP CD4$^+$ thymocytes.[12]

Albinsson et al.[10] obtained thymic samples from infant patients (2 to 4 mo of age) and created an ex vivo culture system whereby human thymic eosinophils were cocultured with human DP, SP CD4$^+$, or CD8$^+$ thymocytes. The researchers reported that when human thymic eosinophils were cocultured with DP thymocytes, there was a significant increase in SP CD4$^+$ thymocytes

relative to DP thymocytes cultured without eosinophils, concluding that thymic eosinophils could be promoting SP CD4$^+$ thymocyte development.[10] Additionally, Albinsson et al. demonstrated that when SP CD8$^+$ thymocytes are cultured with thymic eosinophils there is a significant reduction of SP CD8$^+$ thymocyte frequencies compared with SP CD8$^+$ thymocytes that are cultured without eosinophils, and speculated that eosinophils could be having a suppressive effect on SP CD8$^+$ thymocyte populations by promoting negative selection.[10] The mechanisms by which eosinophils alter thymocyte frequencies is unclear, and because these authors report that human thymic eosinophils lack MHC class proteins,[10] then in this coculture system, eosinophils appear to be impacting thymocyte development/survival independently of these antigen-presenting complexes.

7.2 Eosinophils in thymic maintenance and regeneration

In the thymus, approximately 80% to 90% of the cells occupying the tissue are DP thymocytes. The majority of these DP thymocytes will die due to neglect from the inability to synthesize a functional TCR or will be eliminated through selection. Kim et al.[14] set out to characterize the cell types responsible for clearing dead thymocytes in addition to macrophages, which are known to phagocytize apoptotic thymocytes. Using a model of thymic acute cell death in mice (i.e. administering a single low dose of γ radiation), researchers reported that a large number of neutrophils and eosinophils were recruited to the thymus after a rapid increase of apoptotic cells localized to the cortex.[14] Using mouse models that were void of eosinophils or defective in neutrophil trafficking, the authors showed that clearance of dead thymocytes was greatly impaired in the absence of either of these cell types.[14] However, it should be noted that thymic eosinophils were not found to express traditional markers of phagocytosis (CD68) that were highly expressed by phagocytic macrophages and myeloid dendritic cells, indicating that eosinophils most likely aid in thymic tissue repair as opposed to having a direct role in the phagocytosis of dead cells.[14]

Eosinophils have been shown to be important in regeneration processes after damage to the liver[6] and nervous tissue[64] through secretion of IL-4, and there is evidence they may take on similar roles after thymic damage. Cosway et al.[21] showed that in a model of sublethal irradiation in which mice experience a decline in total thymic cellularity, mice deficient of eosinophils (BALB/c ΔdblGATA mice) were ineffective at regenerating the thymus (i.e. restoring the numbers of total thymic cells, epithelial cell subsets, T cell subsets, and mesenchyme cells). Cosway et al. described a network of type 2 immune cells critical for thymic repair: natural killer T cells stimulate IL-4 receptor signaling in mTEC^{lo} thymic stromal cells to release eotaxin-1, to recruit eosinophils to the thymus in a CCR3-dependent manner.[21] Additionally, the authors reported a positive feedback loop also involving eosinophils that explains another mechanism of thymic restoration: Sca1$^+$ mesenchyme cells are capable of secreting the alarmin IL-33, which causes a transient expansion of ILC2 that cause recruitment of thymic eosinophils in an IL-5–dependent manner.[23] The influx of eosinophils changes the source of intrathymic IL-4 production to shift from primarily natural killer T cells to eosinophils, in which IL-4 stimulates Sca1$^+$ mesenchyme recovery.[23] Moreover, administration of recombinant IL-33[23] and IL-5[21] therapeutically boosts thymic tissue recovery in an eosinophil-dependent manner, and administration of IL-4 negates the requirement for eosinophils altogether.[23]

While thymic eosinophils are critical in regenerating the thymus in a model of sublethal irradiation,[21,23] in a different model of thymic damage induced by myocardial infarction (MI), Shane et al.[65] showed that eosinophils are not essential for thymic regeneration. During MI, high levels of glucocorticoids produced by the adrenal glands reduce thymic weight and cellularity, and cause major thymocyte subsets (double negative 1–4, DP, SP CD4$^+$, and CD8$^+$) to undergo apoptosis at day 7 post-MI.[65,66] The authors then tested whether eosinophils were important in regenerating the thymus at day 14 post-MI when the thymus should be fully recovered, through comparing wild-type and eosinophil-deficient (C57BL/6 ΔdblGATA) mice, and found no significant impairment in recovery in the absence of eosinophils.[65]

Taking these studies on thymic damage together,[21,23,65] it may be that the requirement for eosinophils in thymic regeneration depends on the nature of thymic damage. A noteworthy consideration is that these studies used mice with different genetic backgrounds (BALB/c vs C57BL/6), and this may be a factor impacting the response of and requirement for eosinophils following thymic damage. Indeed, we have previously reported differences in the steady state abundances of thymic eosinophils between BALB/c and C57BL/6 mice.[13] Further work is required to understand how the genetic background and type of thymic damage influence the contributions of eosinophils to thymic recovery.

8. Aging, sex, and pregnancy

The thymus is impacted by many events throughout life such as infection, pregnancy, and age-mediated involution, and the involvement of thymic eosinophils during these major structural changes is not well characterized. With age, the thymus involutes, whereby the stroma undergoes major physiological changes marked by a gradual increase in thymic adipose tissue and a decrease in epithelial tissue.[67] Throsby et al.[12] noted a second peak of thymic eosinophil accumulation at 16 wk of life in mice, and this peak was thought to coincide with a stage of thymic involution. It is possible that eosinophils could be recruited to the thymus to help with maintenance/tissue remodeling as it undergoes these structural changes during adulthood; however, their role in this process is unknown.

Another major shift in thymus structure occurs during pregnancy. During this period, the thymus undergoes a reduction in thymocyte maturation and proliferation, shrinking of the cortex, expansion of the medulla, and overall involution of the thymus itself.[68–70] In a study investigating thymic architecture during pregnancy in mice, Kendall et al.[71] reported the presence and absence of thymic eosinophils during different stages of gestation. The corticomedullary junction and septa exclusively contained fibroblasts during early pregnancy (gestational day 3), but by midpregnancy (gestational day 11–12) these locations were populated with many different immune cell types including eosinophils, while the cortex on the other hand was occasionally populated with a few eosinophils and their abundance was stable throughout pregnancy.[71] The role that eosinophils could be playing in maintaining this dynamic environment during gestation has not been described, but because eosinophils are implicated in thymic regeneration, the possibility remains that they could aid in regenerating the thymus during and potentially after pregnancy.

In studies of thymic eosinophil regulation and function to date, little attention has been given to the potential role of biological sex. We have reported that marginal differences in thymic eosinophil

abundance can be seen between sexes in mice, with female mice appearing to have a greater number of thymic eosinophils than male mice at 2 wk of age.[13] In an interesting study on birds, Höhn[18] reported a cyclic relationship in mallards whereby production of sex hormones and thymic enlargement was inversely correlated. Moreover, this study reported a thymic phenotype exhibited only by female birds during specific months of mating in which "zone strands of eosinophils" divided the lobes of the thymus and eosinophils appeared to be undergoing mitosis.[18] This sex difference seen between thymic eosinophil phenotypes was postulated to be controlled by sex hormones.[18] Sex hormones can have a range of effects on immune cell types including eosinophils. For example, estrogen has been shown to regulate eosinophil migration, adhesion, survival, and degranulation in tissues such as the uterus.[72–74] More studies are required to determine how hormones may affect thymic eosinophils in mammals.

9. Considerations

9.1 Limitations of thymic eosinophil studies in mice

A major limitation of studying thymic eosinophils in mice is the lack of genetic diversity relative to the human condition. Although different genetic mouse strains can be assessed to model genetic differences, this still does not encompass the vast range of genetic deviations possible. In our recent publication comparing eosinophil levels in 2-wk-old C57BL/6J and BALB/cJ mice, we reported that BALB/cJ mice had a lower abundance of thymic eosinophils in both males and females relative to age- and sex-matched C57BL/6J mice.[13] We suspect that this difference is driven by genetic factors rather than by differing microbiomes because administration of a fecal microbiota transplant from a donor C57BL/6J mouse to a pregnant BALBc/J did not increase levels of eosinophils in their pups relative to control mice (pregnant BALBc/J mice receiving a fecal microbiota transplant from a donor BALBc/J mouse).[13]

Another limitation of studying thymic eosinophils in mice is the differences in surface marker expression between human and mouse thymic eosinophils. A previously mentioned example of surface marker discrepancy between mouse and human thymic eosinophils is the expression of MHC class proteins: human thymic eosinophils have been reported to lack MHC protein expression,[10] whereas subsets of mouse thymic eosinophils have been reported to express MHCI classes [12] and II during early life and into adulthood.[13] This could be due to genetic differences between mice and humans. However, in a study looking at the ability of eosinophils to act as antigen presenting cells in vitro, it was noted that ovalbumin-loaded eosinophils subjected to lysosomotropic agents (ammonium chloride and chloroquine) had impaired presentation to ovalbumin-specific T cell hybridomas. Lysosomotropic agents have been shown to disrupt internal antigen processing in B cells[75] and decrease endogenous MHC class II and antigen presentation to T cells in dendritic cells,[76] macrophages,[77] and astrocytes.[78] Ammonium chloride is a commonly used lysosomotropic reagent in red blood cell lysis protocols; therefore, even the method by which human thymic and mouse thymic tissues are processed could result in discrepancies in surface marker expression and functionality of thymic eosinophils.

The final consideration when assessing many mouse studies is whether or not littermate control mice were used when comparing different genotypes (e.g. wild-type mice in comparison with eosinophil-deficient mice). If wild-type mice and transgenic mice are housed in different cages or in different housing conditions (extrinsic factors that could impact the microbiota), it is difficult to parse genotype-driven effects from microbiota-driven effects on phenotypes. The microbiota has been shown to impact T cell development[79] as well as thymic eosinophils themselves,[13] and therefore housing conditions should always be considered when conducting and interpreting mouse experiments.

9.2 Limitations of thymic eosinophil studies in humans

Various congenital cardiovascular diseases require part of the thymus to be cut away to access the heart, and rather than discarding this tissue, researchers have been able to utilize this to study thymic eosinophils.[10,11,24,25] However, this also means that most human studies characterizing thymic eosinophils are limited in that they come from young, pediatric patients that have been diagnosed with various illnesses. It has observed that cortisone levels in the blood of pediatric patients vary (as a consequence of illnesses), and it has been speculated that this could cause variability in the abundance and morphology of thymic eosinophils.[26] This is supported by experiments conducted by Blau[58] showing that cortisone injections in guinea pigs increased both the size and number of Hassall's corpuscles as well as the accumulation of thymic eosinophils within them.

10. Concluding remarks

A growing body of literature is defining the maturation and recruitment process for thymic eosinophils, their phenotypes, and their functional roles in the thymus throughout life. However, there are still many aspects of thymic eosinophil biology that are not understood. What is the role of thymic eosinophils during major physiological events that can impact thymic cellularity and function such as bacterial or viral infections, physical stress, cancer, or pregnancy? Which subsets of αβ thymocytes could eosinophils be interacting with, what mechanisms mediate these interactions, and what is the fate of those thymocytes? Could thymic eosinophils play a role in regulating other cell types that develop in the thymus, such as γδ T cells? What role do thymic eosinophils play during aging-related thymic involution, and are these eosinophils functionally different than those that could be interacting with thymocytes? Do all eosinophils in the thymus undergo local maturation processes, and if so, what signals stimulate this? Do all thymic eosinophils traffic directly to the thymus from the bone marrow? Because eosinophil-depleting therapies are available for disorders involving eosinophilia (e.g. eosinophilic asthma and eosinophilic granulomatosis with polyangiitis), addressing these outstanding questions is essential, such that considerations can be made as to the possible implications that these treatments could have on developing thymocytes, thymic tissue maintenance, repair, or remodeling.

Author contributions

Conceptualization: D.M.G. and L.A.R.; Writing- original draft: D.M.G.; Writing-review, editing, and revision: L.A.R.

Funding

Work in the Reynolds laboratory is supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to L.A.R. (RGPIN-2024-06420). D.M.G. is supported by a Natural Sciences and Engineering Research Council of Canada Canada Graduate Scholarships Doctoral Award (588807-2024).

Conflict of interest statement. The authors have no conflicts of interest to declare.

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