Eosinophils interact with thymocytes and proliferate in the human thymus

Eosinophils are found in the corticomedullary junctions, the medulla, and cortical intraseptal blood vessels of the thymus [1–3]. Both immature cells, for example, eosinophilic promyelocytes and myelocytes, as well as mature eosinophils are present [3], which has led to speculations on the possible occurrence of local proliferation and differentiation of eosinophils in the thymus [3]. The role of the thymic eosinophils remains enigmatic, but two studies entertain the possibility of their involvement in thymocyte selection, by their capacity to induce selective apoptosis of Th1 cells [4] and their association with MHC class I restricted negative selection [5].

In order to unravel the possible immunoregulatory functions of thymic eosinophils, we addressed the following questions: (1) Where are mature and immature eosinophils situated anatomically within the thymus? (2) Do immature eosinophils proliferate and differentiate within the thymus? and (3) Do eosinophils interact with thymocytes? To this end, thymic tissue from 29 children undergoing total or partial thymectomy was investigated (Supporting Information Table S1).

Eosinophil precursors were studied using confocal microscopy by staining thymic cell suspensions for the eosinophil protein galectin-10 and for DNA to visualize nuclear morphology (Fig. 1A). The distribution of eosinophil progenitors was: 16% promyelocytes (>18 μm diameter, rounded nucleus), 38% myelocytes (<18 μm diameter, oval nucleus with indentation), 35% metamyelocytes and band cells, and 11% unclassified. The mature eosinophils were identified by their multinuclei and had a median diameter of 15 μm (25/75%; 12–16 μm). CD34+ eosinophils, that is, the earliest eosinophil precursors [6], were also identifiable in thymic cell suspension (Fig. 1B) and in tissue sections. Every third thymic eosinophil was morphologically immature (Fig. 1C) and about every tenth thymic eosinophil expressed the early precursor marker CD34; 8% according to immunohistochemistry analyses of tissue sections and 17% according to imaging flow cytometry of thymic suspension.

We then assessed if eosinophils can proliferate within the thymus by staining thymic biopsies for the proliferation marker Ki-67. Indeed, every fourth eosinophil in the thymus expressed Ki-67, but only 1.5% of the eosinophils expressed Ki-67 and CD34 simultaneously (Fig. 1D), indicating that most of the proliferating eosinophils had shed this early progenitor marker. No difference in CD34 expression was seen when comparing blood eosinophils with thymic eosinophils, but thymic eosinophils had lower expression of two other maturation markers, Siglec-8 and EMR1 (Fig. 1E and F).

Next, the anatomic distribution of eosinophils was determined by counting the cells in thymic sections using confocal microscopy. Eosinophils were most frequently found in the corticomedullary junctions, followed by the medulla, the cortex, and the interlobular septa. CD34+ eosinophil precursors were mainly localized in the interlobular septa, whereas the Ki-67+ proliferating eosinophils located to the corticomedullary junctions and the medulla (Fig. 1G–L).

Finally, eosinophil interactions with thymocytes were investigated in thymic cell suspension using imaging flow cytometry. The majority (74%) of the eosinophil interactions occurred with CD3+ cells (Fig. 2A). Moreover, eosinophils formed synapses with small mononuclear cells (Fig. 2B). Synapse formation was seen whether or not the thymocytes had been stimulated with CD3/CD28, although there was a tendency toward more frequent synapse formation with the CD3/CD28-activated thymocytes (Fig. 2C). Lastly, we assessed whether thymic eosinophils expressed the T-cell co-stimulatory molecule CD86; eosinophils in the periphery have been shown to express CD86, but not CD80 [7]. Close to half (42%) of the thymic eosinophils expressed CD86 and they were mainly located in the medulla (Fig. 2D and E).

In this study, we provide a more detailed overview of the distribution of thymic eosinophils compared to earlier studies [1, 2]. We confirm the presence of immature eosinophils in the human thymus, and demonstrate that human eosinophils are able to proliferate in the thymus. Our estimate of the proportion of eosinophil precursors in the thymus (30%) is consistent with those of Lee et al., who estimated that up to half of the thymic eosinophils were immature [3]. This is a dramatic figure, unique among the tissues...
Figure 1. Eosinophils of different maturation stages are present in the human thymus. (A) Confocal microscopy images of eosinophil precursors in thymic cell suspension immunostained for galectin-10 (red) and DNA (blue). Eosinophilic promyelocytes with characteristic rounded nucleus (indicated by star) and eosinophilic myelocytes with oval nucleus (indicated by square) are shown. (B) Imaging flow cytometry pictures of thymic eosinophils labeled for MBP, galectin-10 (Gal-10), and CD34. Bar graphs of (C) fractions of morphologically mature and immature eosinophils in thymic cell suspensions and (D) eosinophils that express Ki-67 alone or in combination with CD34 in tissue sections. Flow cytometry median fluorescence intensity (m-FI) values of eosinophil expression of the maturity-related markers (E) Siglec-8 and (F) EMR1 in blood and thymus. (G) Representative image of eosinophil distribution in the thymus; cortex is marked “C,” medulla “M,” corticomedullary junction is indicated by arrows and eosinophils are MBP+ (green). (H) The proliferation marker Ki-67 (red) is expressed within the nucleus of MBP+ eosinophils (green) in thymic sections. (I) Tissue eosinophils labeled for MBP (green) and CD34 (red) displayed separately and in an overlay image. Quantification of the anatomic distribution of (J) thymic eosinophils (K) Ki-67+ eosinophils and (L) CD34+ eosinophil progenitors in the cortex, corticomedullary junctions (CMJ), medulla, and interlobular septa. Bars represent mean ± SEM. Wilcoxon matched-pairs signed rank test was used in (E–F). Included donors/independent experiments for the analyses: (A) 3/3, (C) 3/3, (D) 5/3 and 4/2, (E) 8/8, (F) 9/9, (J) 7/6, (K) 5/3, and (L) 6/4.

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in the body, except for the BM. At this point, we can only speculate why such a large fraction of the thymic eosinophils develop locally. The most immediate interpretation is that the eosinophils require the special environment of the thymus to develop into thymic eosinophils.

Thymic eosinophils have been shown to increase in numbers in a model of acute negative selection of thymocytes in mice [5]. Our finding of direct interactions of human eosinophils with CD3+ thymocytes, including synapse formation and expression of the T-cell costimulatory molecule CD86, is in line with this idea. In contrast, murine thymic eosinophils express neither CD86 nor MHC II [8]. CD86+ human peripheral eosinophils can present antigens to CD4+ T cells and induce them to proliferate in vitro [9]. We also found that the CD86+ eosinophils were mainly located in the medulla, the main site of negative selection [10]. Another possibility is that thymic eosinophils present eosinophil-derived antigens to thymocytes to prevent autoimmune reactions against eosinophils in the periphery.
Figure 2. Thymic eosinophils co-localize with CD3+ thymocytes and form synapses with mononuclear cells. (A) Imaging flow cytometry pictures of eosinophils clustering with CD3+ thymocytes. Cells stained for CD3 (yellow), and galectin-10 (Gal-10, red), Bright Field (BF) images and overlay images are shown. (B) Synapse formation (actin, green) between a galectin-10+ eosinophil (red) and mononuclear cells (blue nuclei) in thymic cell suspensions. Arrow shows one eosinophil forming synapses with three thymocytes. (C) Number of synapses per 1000 galectin-10+ eosinophils in CD3/CD28-stimulated and unstimulated thymic cell suspensions. Wilcoxon matched-pairs signed rank test. (D) Immunohistological staining of an MBP+ eosinophil expressing CD86 (red). (E) Anatomic distribution of CD86+ eosinophils in the cortex, corticomedullary junctions (CMJ), medulla and interlobular septa, mean ± SEM. Included donors/independent experiments for the analyses: (A) 3/3, (C) 5/5, and (E) 5/3.

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