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Human versus mouse eosinophils: "That which we call an eosinophil, by any other name would stain as red"

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Activity Objectives

1. To define the cellular characteristics of human and mouse eosinophils.
2. To differentiate the mechanisms of degranulation of eosinophils in human subjects and mice in the setting of allergic disease.
3. To describe the ways that eosinophils act as a part of the innate immune system and as a regulator of the adaptive immune system.
4. To recognize the similarities between human and mouse eosinophils, which support the translational application of mice as a model for human disease.

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The respective life histories of human subjects and mice are well defined and describe a unique story of evolutionary conservation extending from sequence identity within the genome to the underpinnings of biochemical, cellular, and physiologic pathways. As a consequence, the hematopoietic lineages of both species are invariantly maintained, each with identifiable eosinophils. This canonical presence nonetheless does not preclude disparities between human and mouse eosinophils, their effector functions, or both. Indeed, many books and reviews dogmatically highlight differences, providing

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Terms in boldface and italics are defined in the glossary on page 573.

a rationale to discount the use of mouse models of human eosinophilic diseases. We suggest that this perspective is parochial and ignores the wealth of available studies and the consensus of the literature that overwhelming similarities (and not differences) exist between human and mouse eosinophils. The goal of this review is to summarize this literature and in some cases provide experimental details comparing and contrasting eosinophils and eosinophil effector functions in human subjects versus mice. In particular, our review will provide a summation and an easy-to-use reference guide to important studies demonstrating that although differences exist, more often than not, their consequences are unknown and do not necessarily reflect inherent disparities in eosinophil function but instead species-specific variations. The conclusion from this overview is that despite nominal differences, the vast similarities between human and mouse eosinophils provide important insights as to their roles in health and disease and, in turn, demonstrate the unique utility of mouse-based studies with an expectation of valid extrapolation to the understanding and treatment of patients. (J Allergy Clin Immunol 2012;130:572-84.)

Key words: *Eosinophils, mouse, human, rodent, primate, hematology*

Abbreviations used

CLC: Charcot-Leyden crystal
CMP: Common myeloid progenitor
EAR: Eosinophil-associated ribonuclease
ECP: Eosinophil cationic protein
EDN: Eosinophil-derived neurotoxin
EoP: Eosinophil lineage–committed progenitors
EPX: Eosinophil peroxidase
Gal-10: Galectin-10
GMP: Granulocyte macrophage progenitor
IL-5R: IL-5 receptor
MBP: Major basic protein
MIP: Macrophage inflammatory protein
PMD: Piecemeal degranulation

Preclinical rodent models, mouse models in particular, have been the most widely used in studies attempting to understand the mechanisms underlying human disease. The reasons for this prominence are numerous and varied, as reviewed by Rosenthal and Brown,[1] including the conservation of genome sequence complexity[2,3] and the commonality of biochemical,[4] cellular,[5] and physiologic[6] pathways. In light of these observations, it is surprising that the differences (and, more importantly, not the similarities) between human subjects and mice are often dogmatically highlighted.[7-9] Studies of eosinophils are also subject to this bias, with investigators questioning the value of research on mouse eosinophils and the validity of potential therapeutic options suggested for eosinophil-associated diseases.[10,11] As a consequence, observations suggesting differences in form and function between human and mouse eosinophils are often the focus of many studies and the explanation for the failure of mouse models of human eosinophilic diseases (eg, asthma).

The evolutionary conservation between human subjects (Primata) and mice (Rodentia) is well defined.[12] As a direct consequence, the hematopoietic lineages between these groups

GLOSSARY

5-LIPOXYGENASE: An enzyme that converts arachidonic acid into the first precursor of the cysteinyl leukotrienes.

A PROLIFERATION-INDUCING LIGAND (APRIL): A ligand involved in B-cell signaling and maturation.

ARACHIDONIC ACID: A polyunsaturated fatty acid derived from membrane phospholipids through the action of cytosolic phospholipase A_2.

CASPASES: Enzymes that are cysteinyl proteases that cleave after specific aspartyl residues. Caspases are involved in programmed cell death. A small number of autoimmune lymphoproliferative syndrome cases are caused by mutations in caspase 10.

CD11B: The α chain of complement receptor 3 (CR3), also known as CD11b/CD18 (an adhesion molecule).

CD45RA: A cell-surface marker that is an isoform of CD45. CD45RA is a cell-surface marker for naive T cells. CD45 is essential for T- and B-cell activation.

CHARCOT-LEYDEN CRYSTAL: Colorless crystals that can occur in the sputum after an asthmatic attack.

COSTIMULATORY MOLECULE: Costimulatory molecules are required for optimal cell activation. A commonly discussed costimulatory molecule on T cells is CD28. CD28 binds to 2 ligands expressed on antigen-presenting cells: B7.1 (CD80) and B7.2 (CD86). Ligation of CD28 augments T-cell receptor signals.

DOMINANT NEGATIVE MUTATION: A mutation with a gene product that adversely affects the normal wild-type gene product within the same cell.

FLOW CYTOMETRY: Cells are incubated with mAbs tagged with fluorescent dyes or fluorochromes that emit light when excited by a light source (eg, 488 nm, 633 nm). Cells pass one at a time through the light source, creating 2 types of light emission: scatter and fluorescence. Scatter is measured by using both forward and side detectors. Forward scatter relates to cell size, whereas side scatter is a measure of cell refractivity, which indirectly measures cell granularity.

HETEROCHROMATIC: Relating to heterochromatin, a densely staining chromatin that appears as nodules in or along chromosomes and contains relatively few genes.

MYELOPEROXIDASE: An enzyme that mediates the conversion of H_2O_2 to HOCl and the subsequent killing of phagocytosed bacteria, fungi, and viruses.

NEGATIVE T-CELL SELECTION: Elimination of T cells in the thymus that bind to the endogenous peptide presented by the MHC molecule. These cells are thought to have a high potential for being self-reactive.

NEW WORLD AND OLD WORLD PRIMATES: New World refers to the Americas, whereas Old World refers to Europe, Asia, and Africa. The distinction between these larger groups of primate species arises from their reproductive isolation occurring when South America split off from Africa (approximately 85 million years ago). The ensuing independent evolution of these 2 primate groups has resulted in biochemical, cellular, and morphologic distinctions. The rapid evolutionary events associated with immunologic responses have resulted in significant differences in immunity between New and Old World primates, which are highlighted by unique eosinophil-specific genes appearing in one or the other of these primate groups.

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*Shared reference database of Tables E1 through E3.

are invariantly maintained in type, relative numbers, and general morphology.[13] In particular, the eosinophil lineage–committed granulocyte is a clearly identifiable component of both human and mouse blood.[14] For that matter, identifiable eosinophils are found in virtually every vertebrate species, with even many invertebrates possessing a cell or cells with eosinophilic character.[14,15] The omnipresence of eosinophils nonetheless belies observations that disparities between human and mouse eosinophils exist and that these observed differences might have significance regarding eosinophil activities. However, we believe that in the absence of only a rudimentary understanding of the roles of eosinophils in human subjects, it would be exceedingly shortsighted to focus on small human versus mouse differences, as well as to suggest that these differences are of more significance than the commonalities observed between human and mouse eosinophils.

The goal of this review is to provide both a global perspective and a detailed review of data summarizing what is known about eosinophils in these 2 species, including both similarities and differences. Table I provides the reader with a summary of the concepts addressed in this review of human versus mouse eosinophils, as well as a summation of the extensive supplementary tables linked to this review (ie, titles and numbers of references cited in a given table/topic area; see Tables E1-E9 in this article's Online Repository at www.jacionline.org). Our objectives will be 2-fold: (1) to provide an easy-to-use reference guide to important studies contrasting the hematopoietic origins, morphology, proposed effector functions, and role of eosinophils in human subjects and mice and (2) to demonstrate that although differences exist, more often than not, their consequences are unknown and do not necessarily reflect inherent differences in granulocyte function but instead species-specific variations. More significantly, despite these differences, the vast similarities between human and mouse eosinophils tell us far more regarding the role of eosinophils in health and disease.

EOSINOPHILOPOIESIS: HEMATOLOGIC ORIGIN AND DEVELOPMENT/MATURATION

The generation of terminally differentiated eosinophils from pluripotent hematopoietic stem cells follows a similar path in human subjects and mice (Fig 1). Specifically, the isolation of a progenitor population of eosinophils in mice has led directly to the recent identification of eosinophil lineage–committed progenitors (EoPs) in human subjects.[16,17] In both species hematopoietic stem cells in the bone marrow progress through the multipotent progenitor stage followed by the common myeloid progenitor (CMP) stage before branching into EoPs in human subjects or through granulocyte macrophage progenitors (GMPs) and into EoPs in mice (Fig 1, reviewed by Uhm et al[18]). The surface phenotype used to identify EoPs in human subjects was Lin^-, IL-5 receptor (IL-5R) α^+, $CD34^+$, $CD38^+$, IL-3Rα^+, ***CD45RA***$^-$ whereas in mice it was Lin^-, IL-5Rα^+, Sca^-, $CD34^+$, $ckit^{lo}$.[16,17] Mori et al[17] have revised the human CMP population to account for an IL-5Rα^+ population of CMPs (ie, EoPs) from which eosinophils can develop. Furthermore, the fact that human GMPs, unlike mouse GMPs, do not differentiate into eosinophils might suggest that a revision of the human GMP definition is possible as well.

Transcription factors important to eosinophil development are shared by both species. In particular, GATA-1 and C/EBP-α expression are critical to eosinophil differentiation. Deletion of GATA-1 or C/EBP-α in mice results in loss of the eosinophil lineage. Expression of a ***dominant negative mutation*** form of GATA-1 in human cord blood progenitor cells prevented eosinophil differentiation in culture, and ectopic expression of either GATA-1 or C/EBP-α in cord blood progenitors led to eosinophil differentiation.[19] Promisingly, EoPs from both species display a similar pattern of expression of GATA-1 and C/EBP-α, as well as other transcription factors thought to play key roles in eosinophil differentiation in both species, including PU.1, FOG-1, and GATA-2.[16,17]

Cytokines important to the survival, expansion, and terminal differentiation of eosinophils from EoPs include IL-3, IL-5, and GM-CSF. Of these, IL-5 is the most specific to eosinophil development in both species.[20] Accordingly, IL-5 effectively supports proliferation and survival of eosinophils from bone marrow culture in human subjects[21] and mice.[22]

Once eosinophils have matured, they either remain in the marrow or exit into the circulation and then quickly "home" to selected compartments/tissues. Under inflammatory conditions, eosinophil progenitor levels are increased in the marrow and are found in the circulation, as well as in inflamed tissues of each species. Moreover, inflammation can also promote eosinophilopoiesis at extramedullary sites in both human subjects and mice.[23]

In summary, evolutionary conservation of eosinophilopoiesis among mammals ultimately represents an experimental strength as investigators strive to understand the significance of hematologic changes occurring in mouse models.

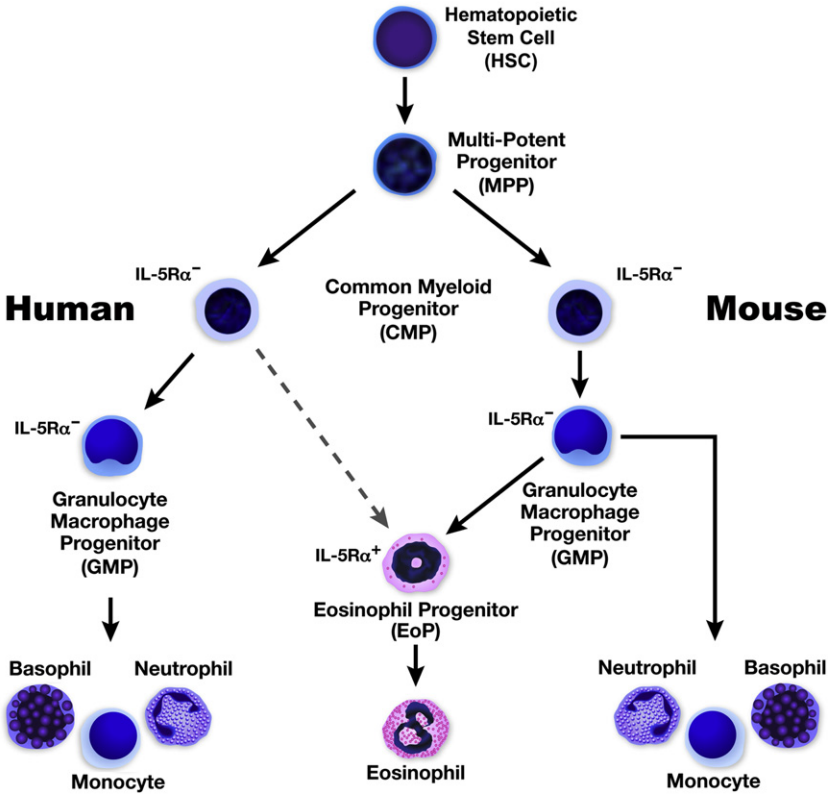


FIG 1. Hematopoietic differentiation pathways leading to eosinophils in human subjects versus mice.

DEFINING CHARACTERISTICS OF MATURE EOSINOPHILS IN PERIPHERAL CIRCULATION

Cell morphology

Early in the microscopic examination of human blood and that of many other species, certain similarities of the formed cellular elements became apparent. Dyes, primarily used to color fabrics (eg, silk, wool, and cotton) and writing fluids, were applied to blood films to facilitate visualization. Cytoplasmic granules of a subset of nucleated blood cells of both human subjects and mice reacted with acidic aniline dyes. During the latter part of the 19th century, these granulated leukocytes were named "eosinophils" because of the popular use of dye sets containing eosin Y. Romanovski (cited frequently as Romanowsky) was the first to mix the acidic aniline dye eosin Y with azure B to stain blood cells,[24] followed by Giemsa (1902)[25] and Wright (1902) stain,[26] each modifying the proportions and methods of preparation of the eosin Y:azure B or azure A+B (a.k.a. azure I) mixture.

Staining properties using Romanowsky dye sets

By using light microscopy, Romanowsky-stained eosinophil granulocytes of both human and mouse eosinophils have been examined in samples from a variety of sources (eg, marrow, circulating blood, nasal swabs, aspirates, bronchoalveolar lavages, and sputum). Although qualitative differences might exist, the similarities between these species are remarkable (Fig 2 and see Walker et al[13] for source references on comparative morphology). Eosinophil numbers are within comparable ranges (1% to 3% of total white blood cells) in blood of healthy members of both species. The eosinophils differ only slightly in size, with human eosinophils being somewhat greater in diameter (12-15 μm vs 9-12 μm, respectively). Definitive granules of human eosinophils are also slightly larger and commonly more densely packed in the cytoplasm. Human eosinophils also stain a more vibrant magenta with eosin relative to mouse eosinophils because of the collectively higher cationic change of the human granule proteins (as reviewed below). The nuclei of human and mouse eosinophils are both polymorphic in character. Specifically, the nuclei of EoPs are converted during differentiation from spherical structures to elongated and somewhat cylindrical (band and stab forms) shapes. These nuclear transformations continue, and eventually the nuclei of the mature metamyelocytes segment into multiple lobes (human eosinophils) or, as in the case of mouse eosinophils, to ring-like structures that also segment, although less so, before their exit into peripheral circulation. Nuclei of human eosinophils are typically more ***heterochromatic*** than those of mouse eosinophils, the significance of which is not understood.

Electron microscopic morphology

The circulating mature eosinophils found in the peripheral blood of both human subjects and mice share a common and distinct electron microscopic morphology.[27,28] This common electron microscopic morphology is highlighted by the unique appearance of the polymorphonuclear changes that often appear as multiple nuclear compartments within the cytoplasm reflective of the multilobed (human subjects) or ring-like structure (mice) of the nucleus traversing in and out of the section's plane. Moreover, the identification of the abundant secondary granules in the eosinophils of either species is based primarily on these electron microscopic observations. These assessments invariably show the

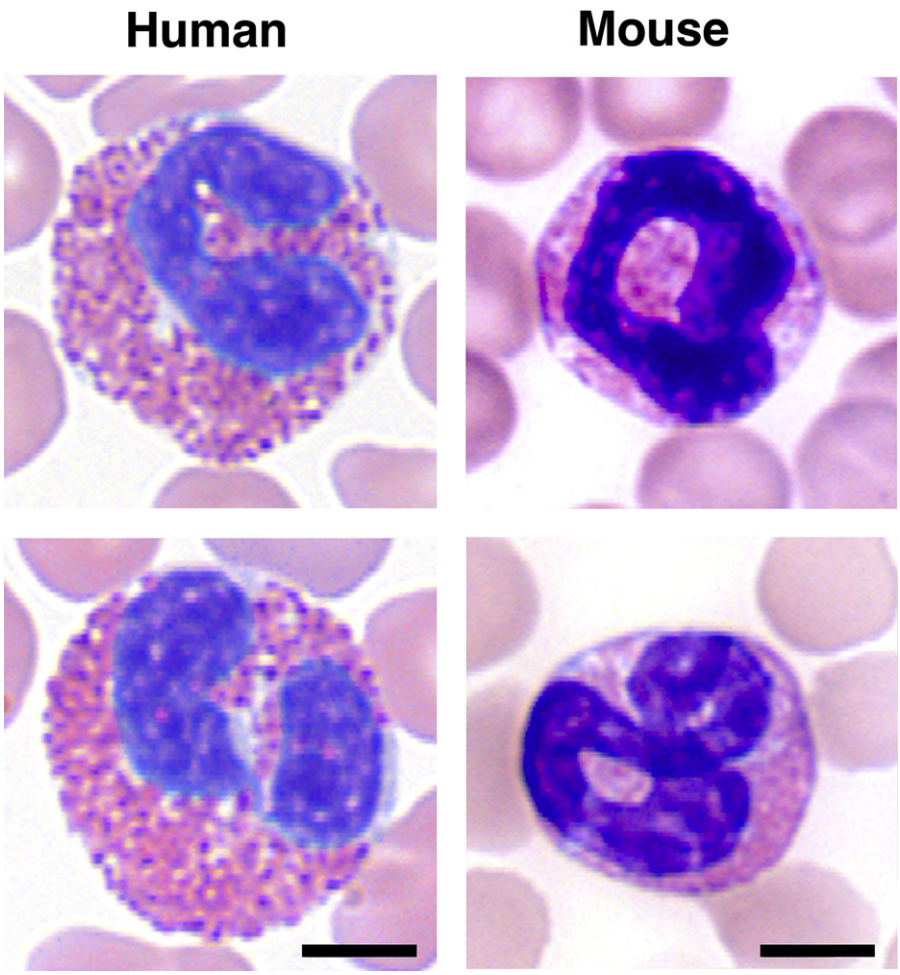


FIG 2. Human versus mouse eosinophils stained with Romanowsky dye sets reveal remarkable similarities in cell morphology punctuated by subtle differences, the significance of which is not yet fully understood. Representative eosinophils were identified from stained blood films of a human subject and a BALB/CJ mouse and shown in comparison. *Scale bars* = 5 μm.

presence of an electron-dense crystalline core within the secondary granules of both human subjects and mice that is primarily composed of major basic protein (MBP) 1[29-31] and an electron-translucent matrix comprised of the other abundant eosinophil granule proteins.[29,30,32] It is noteworthy that studies in both species have also identified a host of other atypical proteins stored in the secondary granules of eosinophils, including an array of cytokines and chemokines.[33-35]

Cell-surface inventory

The advent of ***flow cytometry*** has provided investigators with an unprecedented ability to identify/characterize white blood cells by their expression of cell-surface molecules. In part, this is due to an association of these cell-surface molecules with not just the identification of a white blood cell type but also its activation status and other phenotypic features that permit various subclassifications. The identification of eosinophils by means of flow cytometry at its most basic level relies on both the physical nature of the cell and specific cell-surface molecules. It is important to note that many of these molecules are found on other cell types as well (see Tables E1-E3). On a macro level, both human and mouse eosinophils are high in granularity and therefore "gate" in flow cytometric plots as side-scatter^{hi}. Thus in addition to the use of specific cell-surface markers, eosinophils in either species are easily identified. For example, in both human subjects and mice, eosinophils can be described simply as side-scatter^{hi} cells staining positive for IL-5Rα and negative for the lineage markers CD4, CD8, B220, and CD19. Other markers, including CCR3 (human subjects/mice), Siglec-8 (human subjects)/Siglec-F (mice), EMR1 (human subjects)/F4/80 (mice), and ***CD11b*** (human subjects/mice), are often used to further ensure identification of eosinophils in either human subjects or mice. Interestingly, CCR3 is specific for eosinophils in mice, yet can also be found on other cells, such as T cells, in human subjects. Moreover, Siglec-F in mice is present on eosinophils and alveolar macrophages, whereas Siglec-8 is present on human eosinophils and mast cells. Similarly, F4/80 is expressed by mouse eosinophils and macrophages, whereas the human ortholog EMR1 is highly specific for human eosinophils.

Some useful identification markers are not present on human cells but are highly used for mouse white blood cell characterization. In the mouse the Gr1 cell-surface marker subtype Ly6-G recognizes eosinophils and neutrophils, with eosinophils being Gr1^{lo} and neutrophils being Gr1^{hi}. Human subjects do not express Gr1 and instead follow a pattern whereby resting blood

eosinophils would be $CD14^{lo}/CD16^{lo}$ and then $CD14^{med}/CD16^{med}$ on activation. These changes in expression could have consequences in various studies given that peripheral blood eosinophils are generally isolated away from neutrophils by mean of magnetic depletion of $CD16^{hi}$ neutrophils.

As just described, cell-surface markers are also an indicator of activation status of the cell. In particular, eosinophils exposed to activating agents, such as platelet-activating factor, increase their expression of CD69 in both human subjects and mice. Additionally, mouse and human eosinophils will upregulate chemokine receptors (eg, CX3CR1), ***costimulatory molecules***, and antigen presentation molecules (eg, CD86 and MHC class II) on activation. The study of eosinophil surface markers is expanding but nonetheless remains behind studies of other leukocytes, such as neutrophils and mononuclear cells, including monocytes, lymphocytes, and dendritic cells, in both human subjects and mice (see Table E2 vs Table E3). It is likely that similar to the increases in subclassifications associated with other white blood cells, eosinophil subtype classifications will grow significantly in the next few years.[36] We anticipate that this growth in eosinophil subtype classifications will likely occur in both species, with more similarities rather than differences noted.

Evolutionary conservation, divergence, or both of characteristic eosinophil-associated genes/proteins

Discussions of eosinophil-associated genes/proteins invariably focus on the genes encoding either the prominent groups of secondary granule proteins (ie, eosinophil MBPs, eosinophil-associated ribonucleases [EARs], and eosinophil peroxidase [EPX][37]) or the abundant and primarily cytoplasmic protein that composes the ***Charcot-Leyden crystal*** (CLC), a structure characteristically found at sites of eosinophil-mediated inflammation.[38] The commonalities and lack thereof of these genes/proteins between human subjects and mice will be used to highlight general principles and concepts. However, it is important to keep in mind that the significance of either the reported differences or similarities of these genes/proteins between human subjects and mice is often obscured by the confounding lack of a clear understanding of the function of the encoded proteins (MBPs and CLC), the role the identified protein function has in eosinophil-mediated activities (EARs and EPX), or the relative importance of degranulation and the site-specific release of these proteins.[33] In addition, it is also noteworthy that other eosinophil-associated genes/proteins of significance exist and that in many cases these are noted in other areas of this review.

Eosinophil MBPs. Orthologous gene pairs (MBP-1 and MBP-2) are found in both human subjects and mice.[39-42] In both species the abundance of the protein encoded by MBP-1 is the highest on a molar basis among the eosinophil secondary granule proteins and is greater than an order of magnitude higher relative to the protein encoded by the MBP-2 gene.[41,42] Nonetheless, significant differences between human subjects and mice exist in terms of both the expression of these genes and the biochemistry of the encoded proteins. Human MBP-1, unlike mouse MBP-1, is transcribed from multiple promoters.[43] Human and mouse MBP-1 appear to be predominantly expressed in eosinophils (composing the electron-dense cores characteristic of the eosinophil secondary granule). However, in human subjects (unknown in the mouse) expression extends to other leukocytes.[44,45] In human subjects MBP-1 is highly cationic, whereas MBP-2 has lost this cationic character.[42] In contrast, these 2 proteins retain their cationic character in the mouse.[41] Finally, in human subjects expression of MBP-1 extends to nonleukocytes, such as placental X cells, which leads to the accumulation of the unprocessed "pro"-form of this protein during pregnancy.[46] This placental expression and serum accumulation of pro–MBP-1 does not occur in the mouse.[41]

EARs. The EARs are a rapidly evolving group of genes that encode multiple family members stored in the electron-translucent matrix of the secondary granules of both human (eosinophil cationic protein [ECP][47] and eosinophil-derived neurotoxin [EDN][48]) and mouse (Ear-1, Ear-2, Ear-6/7, and Ear-5/11[49,50]) eosinophils. These stored ribonucleases are second only to MBP-1 in abundance on a molar basis in both species. ECP is a very cationic protein with weak ribonuclease activity[48] found only in human subjects and other ***Old World primates***.[51] In comparison, EDN is a nominally cationic protein that has a strong ribonuclease activity,[51] with orthologs present in both Old World and ***New World primates***.[51] The mouse EARs are not orthologous with human ECP and EDN and instead represent distant paralogous genes the expansion of which occurred well after the divergence of Primata and Rodentia.[50] In the mouse all of the EARs are nominally cationic proteins, with each retaining strong ribonucleolytic activities.[49,50] In both human subjects and mice, these ribonucleases are stored as mature proteins retaining activity in eosinophil secondary granules. Moreover, in both species this family of genes is expressed in many cell types other than eosinophils, including other leukocytes[52,53] and nonhematopoietic cells.[49,54]

EPX. EPX genes in human subjects and mice are orthologous genes encoding proteins that display significant peroxidase enzymatic activities and an extraordinarily high level of sequence identity at the amino acid level (>94%[55,56]). In both species EPX is found as part of a closely linked leukocyte-specific pair of peroxidase genes (***myeloperoxidase***-EPX) the duplication event of which occurred before the divergence of Primata and Rodentia.[12] Similar to the encoded EARs, EPX in both human subjects and mice is stored as a mature functional protein in the electron-translucent matrix of eosinophil secondary granules and is the most abundant proteins (by mass) found in the granules of both species. Among all of the eosinophil granule proteins in either human subjects or mice, EPX appears to be the most eosinophil specific, with no published reports demonstrating expression in other leukocytes or nonhematopoietic cell types.

CLC protein. Human CLC protein was originally identified as a lysophospholipase expressed predominantly in the cytoplasm but also present in the primary granules of eosinophils.[57] However, subsequent studies have demonstrated that human CLC is not a lysophospholipase and instead is a member of the larger galectin family that is also described as galectin-10 (Gal-10).[38] Studies of CLC/Gal-10 in human subjects demonstrated that it is one of the most highly expressed genes in eosinophils[45] and that this expression was not limited to eosinophils, with transcripts also noted in regulatory T cells.[58] The activities mediated by CLC/Gal-10 in either eosinophils or T cells are unclear, and in turn, the role or roles of CLC/Gal-10 in human subjects remain obscure. Surprisingly, despite its prominent level of expression in eosinophils and the relative conservation of the galectin gene family between human subjects and mice, genome sequencing has demonstrated unambiguously that CLC/Gal-10 is absent from the mouse genome,[3] which is consistent with the lack of

reports in the literature noting the presence of this structure in mouse models of human disease.

EOSINOPHIL LOCATION AND RELATIVE ABUNDANCE AT HOMEOSTATIC BASELINE AND DISEASE

The 20th century saw many significant attempts to expand on the observations of Paul Erlich[59] and correlate eosinophil infiltration of organs/tissues with health and disease, such as the work of Huber and Koessler.[60] These ongoing studies continue today with ever-increasing scope and complexity.[61] A commonality that surrounds all of these studies (despite the hyperbole associated with some reviews in the literature[9,10]) is that the identification of eosinophils in tissues/organs during health and disease has been strikingly similar between human subjects and mice. For example, both human subjects and mice have similar peripheral blood and marrow distribution of eosinophils relative to other white blood cells; as such, eosinophils comprise approximately 2% of the peripheral white blood cells and approximately 8% of bone marrow leukocytes.[13] Eosinophils are also found at homeostatic baseline of otherwise healthy human subjects and mice in the gastrointestinal tract (ie, stomach to rectum), thymus, secondary lymphoid tissues, uterus, and adipose tissue.[61] The specific and unique role or roles of eosinophils in the tissues/organs of healthy subjects in both species is the subject of current debates and investigations. However, the commonality of this tissue distribution between human subjects and mice suggests eosinophils contribute to tissue/organ-specific pathways mediating the maintenance of homeostasis[15] and that these roles have been conserved since the radiation of the major extant mammalian orders.[12]

An equally provocative conservation between human subjects and mice occurs when examining the relative increase in eosinophil numbers and location as part of the changes associated with different diseases (see Table E4). In many cases the specific and unique role or roles of eosinophils in these tissues remains to be defined; yet again, the overwhelming consensus is that far more similarities between human subjects and mice exist relative to small variations that have been observed (many of which remain unclear, unresolved, or both).

Interestingly, this list of eosinophil-associated diseases in human subjects has expanded dramatically over the last 2 decades. Thus eosinophils are now linked with an expansive number of diseases in human subjects beyond simply parasitic infections and allergic diseases, such as asthma.[62] More importantly, as documented in Table E4, this expanding number of eosinophil-associated human diseases has been accompanied by a concomitant increase in mouse models of human disease with similar eosinophil associations. This commonality again suggests an underlying conservation of eosinophil effector functions among mammals. In turn, this lack of substantive differences between human subjects and mice raises expectations for the utility and ultimately the validity of mouse models in studies of eosinophil-associated diseases in human subjects.

EOSINOPHIL TRAFFICKING AND RECRUITMENT

Inflammatory cytokines are released in response to tissue injury, infection, or both, stimulating the surrounding cells to produce adhesion molecules and chemotactic factors that signal the recruitment of various leukocytes, including eosinophils. The movement of eosinophils out of the circulation to sites of inflammation is a well-orchestrated process that involves adhesion to the vascular endothelium, diapedesis into surrounding tissues, and trafficking to inflammatory sites. This process has been well studied in both human subjects and mouse models, with very little disparity. Tables E5, E6, and E7 provide details available regarding the similarities/differences between human subjects and mice associated with eosinophil diapedesis and recruitment into inflamed tissues. Specifically, they address similarities/differences in cell adhesion molecules (see Table E5), chemokines and their corresponding receptors (see Table E6), and nonchemokines and their receptors (see Table E7). As is apparent from these tables, there are only a few differences between human and mouse eosinophils with respect to trafficking and recruitment. Despite this overwhelming similarity in human versus mouse recruitment/trafficking, several areas of debate and/or notable difference in eosinophil chemotaxis exist.

First, although CCR3-mediated chemotactic events between human subjects and mice are conserved, differences exist in the expression of the various eotaxin chemokines. There are 3 known human eotaxin chemokines (eotaxin-1 [CCL11], eotaxin-2 [CCL24], and eotaxin-3 [CCL26]) expressed in response to inflammatory events. Mice, on the other hand, only express eotaxin-1 (CCL11) and eotaxin-2 (CCL24) and possess only an eotaxin-3 (CCL26) pseudogene (ie, a nonfunctional gene). In addition, mice do not display eosinophil chemotaxis in response to human eotaxin-3.[63]

Second, RANTES is an eosinophil agonist chemokine (CCL5) that is also involved in recruitment of monocytes, eliciting effects through interactions with either CCR3 or CCR1 receptors. In human subjects RANTES (CCL5) binds directly to appropriate receptors on eosinophils to signal their recruitment.[64] Mice express a RANTES ortholog (CCL5), and *in vivo* experiments appear to show that it also elicits the recruitment of eosinophils.[65-68] However, purified mouse eosinophils do not chemotax in direct response to RANTES (CCL5), likely because of the inability of mouse RANTES (CCL5) to bind to mouse CCR3.[63] This suggests that although RANTES (CCL5) might mediate eosinophil chemotaxis in the mouse, it occurs through an unknown mechanism or mechanisms of activation mediated by other concurrent inflammatory pathways. Thus species-specific differences in cytokine/chemokine-mediated trafficking events might exist in detail, but other selective pressures have maintained a degree of common functionality.

Third, as noted above, small molecule–mediated chemotaxis of eosinophils is a central component of recruitment and trafficking, particularly products of ***arachidonic acid*** metabolism, such as leukotriene B_4.[69] However, many other lipid mediators with very robust eosinophil chemotactic properties have been identified, with 5-oxo-ETE as a representative example. This lipid mediator is generated from its precursor, 5-HETE, through oxidation in the ***5-lipoxygenase*** pathway of arachidonic acid metabolism. In human subjects it is a strong chemoattractant that specifically binds to the oxoeicosanoid receptor 1, which is highly expressed on eosinophils.[70-73] In mice its role in eosinophil chemotaxis is not yet fully elucidated. Specifically, studies have not demonstrated a strong chemotactic response (our unpublished observations), possibly because of the lack of the specific receptor (ie, oxoeicosanoid receptor 1) that has yet to be identified on the cell surface of mouse eosinophils.[74]

EOSINOPHIL DEGRANULATION AND MEDIATOR RELEASE

Eosinophil degranulation

Human and mouse eosinophils are, by definition, granulocytes, and for obvious reasons, the process of degranulation (ie, the extracellular release of granule contents) has been suggested to be singularly important to eosinophil effector function or functions. Specifically, after 1 or more events characterized as "priming" or "activation," eosinophils are capable of rapid and stimulus-specific release of intact granules, preformed granular contents, or both independent of the classical ER-Golgi secretion pathway. Clinical studies, *in vitro*/*ex vivo* experimentation, and assessments of mouse models of human disease suggest that 4 mechanisms, pathways, or both leading to degranulation exist that can vary between human subjects and mice (see Table E8). These mechanisms include (1) classical exocytosis (ie, the release of granule contents through the fusion of individual granules and the plasma membrane); (2) compound exocytosis (ie, homotypic fusion of multiple secretory granules to form "supergranules" before fusion with the plasma membrane); (3) piecemeal degranulation (PMD; ie, gradual stepwise release of granule contents through secretory vesicles budding, mobilizing, and fusing with the plasma membrane); and (4) cytolytic release of otherwise intact granules (ie, the release of otherwise intact cytoplasmic granules as a consequence of rupturing the plasma membrane as part of necrotic cell death).

Studies of both human subjects and mouse models have demonstrated that PMD is a common mechanism of eosinophil degranulation. Secretory vesicles fusing with the plasma membrane have been detailed elegantly in several *in vitro*/*ex vivo* studies using human eosinophils.[75-80] PMD in human subjects has also been shown to occur in response to defined environmentally relevant stimuli (eg, antibody-mediated cross-linking of Fc receptors[81]). However, such demonstrations are limited when investigating mouse eosinophils. Although the available data suggest that mouse eosinophils are capable of undergoing PMD, examples of this phenomenon in the mouse are rare by comparison and only mechanistically suggestive. For example, exposure of mouse eosinophils *in vitro* to 1 or more agonists appears to elicit the piecemeal release of eosinophil granule proteins,[82,83] and in some cases these activated eosinophils display ultrastructural changes detectable by using electron microscopy suggestive of PMD.[83] *In vivo* studies of mouse disease models also provide evidence suggestive of PMD, including assessments of eosinophils recruited to the lung in response of allergen provocation[84,85] and eosinophils accumulating in the lung as a consequence of constitutive expression of IL-5 and eotaxin-2 (CCL24).[86]

Studies of patients highlight the surprisingly wide scope and extensive character of ECL as a mode of release of human eosinophil granule components. The extracellular deposition of otherwise intact eosinophil secondary granules within tissues are readily detectable in patients with allergic asthma,[87] rhinitis,[88] atopic dermatitis,[89] acute lung injury,[90] and eosinophilic esophagitis.[91,92] The commonality of this phenomenon has led investigators to give a name to these released and largely intact eosinophil granules: clusters of free eosinophil granules.[93] Indeed, recent studies in both human subjects and mice have suggested that these extracellular granules might even function autonomously, responding to physiologically relevant stimuli in their tissue microenvironment.[83,94] However, in contrast to human subjects, documented cases of ECL occurring in mouse models of human disease are very limited, and even these cases are more subjective interpretation than demonstrable evidence of this mechanism.[86,95]

Collectively, the data show that the release of eosinophil granule proteins (ie, degranulation) is a phenomenon occurring in both human subjects and mice. Nonetheless, species-specific mechanisms achieving this degranulation *in vivo* are also evident. This dichotomy is highlighted as the easily observable eosinophil degranulation that occurs in human asthmatic patients[96,97] in contrast to only sporadic reports of nominal (if any) eosinophil degranulation occurring in the lungs in mouse models.[31,32,82,86,98-100] These observations demonstrate that the responses of human and mouse eosinophils to physiologic stimuli in all cases are not necessarily the same, and in regard to asthma studies, these differences have led some investigators to question the relevance/importance of mouse models.[10,11] Unfortunately, the resolution of this and other debates regarding the utility of mouse models of human disease has been slow in coming because the significance of these species-specific differences has often remained unclear. Moreover, the role of degranulation itself in eosinophil effector function or functions in either human subjects or mice is still debatable and awaits a more complete experimental definition.[33]

Eosinophil mediator release

The importance and critical character of the release by eosinophils of various mediators is highlighted by the wealth of outstanding reviews with in-depth discussions published recently.[83,95,101-105] They describe and review efforts to elucidate triggers, mechanisms, and subsequent pathways resulting from mediator release in the context of homeostasis and disease pathology. Table E9 is a comprehensive summary of mediators secreted from eosinophils, stimuli/conditions that cause/aid this release, and exhaustive references to the respective studies. This summary is inclusive of both *in vitro*/*ex vivo* studies of isolated populations of eosinophils (thus avoiding ambiguity as to the source of the mediators), as well as examples of eosinophil degranulation and mediator release *in vivo*. For the most part, the available studies do not necessarily allow exact comparisons between human and mouse eosinophils; few complementary studies of these granulocytes exist using the same experimental conditions, agonist stimuli (ie, in most cased responses were achieved with different secretagogues), or identical *in vivo* provocations (a rare exception is Meeusen and Balic[106]). Despite the comparative difficulties, it is extraordinarily informative that even though experimental conditions have often varied in detail, the composition of granule proteins and cellular mediators released from either human or mouse eosinophils in response to comparable stimuli have largely overlapped.

As shown in Table E9, and as discussed earlier, human and mouse eosinophils are each capable of (1) secreting cationic proteins from their secondary granules that are often orthologous in origin; (2) releasing reactive oxygen species on priming/activation; (3) secreting cytokines, chemokines, growth factors, and lipid mediators as part of innate and acquired immune responses/inflammation; and (4) releasing mitochondrial DNA and other antibacterial agents as part of innate host defense. The mediators and secretagogues studied in one species were

often not studied in the other, and thus it is noteworthy that apparent human versus mouse variations are not necessarily differences but simply the lack of overlapping datasets (see Table E9). For example, the secretion of β-glucuronidase, soluble CD16, ENA-78, GM-CSF, IL-8, kynurenins, macrophage inflammatory protein (MIP) 3, matrix metalloproteinase 9, nerve growth factor, osteopontin, stem cell factor, and TGF-α has been studied extensively in human subjects. However, similar studies have not been reported in the mouse or at best have only tangentially been addressed. Alternatively, the release of C10, ELC, IL-17, IL-2, interferon-inducible protein 10, monocyte chemoattractant proteins 1 and 5, macrophage-derived chemokine, monokine induced by IFN-γ, MIP-1α, MIP-1γ, MIP-2, thymus and activation-regulated chemokine, and TGF-β secretion was studied in far more detail in mouse eosinophils in comparison with their human counterparts.

It is also instructive to recognize that the studies available in the literature have noted species-specific differences in mediator release between human and mouse eosinophils. These differences include secretagogues that commonly initiate degranulation in human eosinophils (IL-5, IL-3, GM-CSF, and eotaxin-1 [CCL-11]) but have little to no effect on mouse eosinophils. In addition, some stimuli, such as platelet-activating factor, elicit the secretion of IL-9, IL-13, IL-1 receptor α, basic fibroblast growth factor, RANTES, and platelet-derived growth factor β from human eosinophils but did not have similar effects on mouse eosinophils derived *ex vivo* from bone marrow cultures.[106] As is often the case in comparisons of human and mouse eosinophils, the significance (if any) of differences noted in mediator release are unknown. Thus although these differences exist and are experimentally repeatable, they might represent only nominal variations between distinct mammalian species and not necessarily fundamental divergences in eosinophil effector functions.

EOSINOPHILS AS REGULATORS OF THE IMMUNE MICROENVIRONMENT

As noted earlier, hematologic systems and immune pathways are very similar between human subjects and mice. This is inherently evident by the use of the mouse as a model of human disease not only in academic research but also in pharmaceutical industry studies. However, only recently have studies of both human and mouse eosinophils in health and disease enabled an appreciation of the eosinophil as a specific immune regulatory cell on par with the functionality of other regulatory cells (eg, dendritic cells).[15,103,107,108]

Eosinophils from both human subjects and mice are similar in their capacities to function as both innate cells and regulators of adaptive immunity. This includes the expression of surface markers of immunologic relevance (see Table E1), the expression of immune-modulating cytokines (T_H1: IL-12 and IFN-γ; T_H2: IL-4, IL-5, IL-9, IL-13, IL-25; proinflammatory: IL-6, IL-1β, and TNF-α; suppressive: TGF-β, IL-10, and indolamine-2,3,oxygenase[35,109]), proteases (eg, matrix metalloproteinase 9[110,111]), and lipid mediators (eg, leukotrienes and prostaglandins[112,113]), the activation of receptors eliciting innate responses to select pathogens (eg, Toll-like receptors[114,115] and proteinase-activated receptor 2[116-118]), the generation of reactive oxygen species,[119] and the release of genomic/mitochondria/DNA.[120] Some of these immune mediators have been described in human subjects but do not exclude their expression in mice. Thus many of the differences between human subjects and mice are either a result of insufficient research, an inability to assess eosinophil activities in human subjects, or true interspecies variations.

The role of eosinophils as immune regulators of the adaptive immune system was originally realized on the isolation of eosinophils from human subjects that were found to have increased MHC class II and costimulatory receptor expression.[121-123] Mouse models enabled the elucidation of this function and demonstrated a similar capacity to process and present antigens,[124,125] migrate to the secondary lymph nodes,[126] and induce cytokine release from T cells.[127,128] Through the use of eosinophil-deficient animals, eosinophils were demonstrated to modulate directly the local immune polarization of T_H2 versus T_H1/T_H17 T-cell responses.[129] These complex systemic pathways are not possible to measure in human subjects with current methodologies but likely occur based on correlations found between eosinophils and immune polarization in various disease states, such as viral exacerbation in asthmatic patients,[130] transplant rejection,[131] and autoimmune diseases,[132] as well as potentially related/similar inflammatory events (eg, inflammatory bowel disease[133]).

Additional immune responses that have only been demonstrated to date in the mouse but are not excluded to occur in human subjects include the role of eosinophils in plasma B-cell survival through the release of IL-6 and ***a proliferation-inducing ligand (APRIL)***.[134] This might have particular significance in human subjects, who also rely on IL-6 and a proliferation-inducing ligand for plasma B-cell survival.[135] Moreover, in the mouse glucose homeostasis has also been demonstrated to be mediated by eosinophil-dependent immune regulation of macrophages in adipose tissue.[136] These immune regulatory activities of eosinophils on macrophages in mice might represent a novel inflammatory process linked to metabolic diseases of human subjects, in whom inflammatory macrophages are known to be significant mediators of disease.[137] In addition, mouse eosinophils have been shown to modulate immune environments for remodeling and repair in health and disease, such as reproductive development[138,139] and neuronal branching and growth.[140] In the latter example parallels between human and mouse eosinophils are highlighted by similar capabilities to express neuromediators, suggesting these mechanisms are translational.[141]

Eosinophil associations with underlying immune events in a larger context have also been described in both human subjects and mice. For example, human and mouse eosinophils have been shown to localize to the thymus and are hypothesized to have a contributory role in the process of ***negative T-cell selection***,[142,143] T_H2 polarization of the thymus,[142,144] or both. In both human subjects and mice, eosinophil Toll-like receptor activation is associated with antiviral activities.[145-147] Moreover, eosinophil immune/remodeling pathways are likely similar between human subjects and mice in response to cancer and transplant rejection, particularly because eosinophils are found in parallel tissue sites in equivalent numbers (see Table E4).

In summary, just as dendritic cells, T lymphocytes, macrophages, B cells, and mast cells, for example, have similar immune activities between human subjects and mice, eosinophils from these species also appear to display similar immune-mediating functions. Differences that do arise are likely due to evolutionary events that result in paralogous functions or alternative pathways leading to the same end point. Occasionally, evolutionary divergences do exist, yet they appear to be limited in number and

represent only a minor portion of the total immune response or responses. Thus the further eosinophils are studied in both patients and mouse models of human disease, the more similar their respective immune activities and functions become apparent.

THE END GAME: EOSINOPHIL TURNOVER

Eosinophil survival and death are regulated by the local immune microenvironment. The cytokines IL-3, IL-5, and GM-CSF generally enable survival of eosinophils in both human subjects[148] and mice.[149] Withdrawal of these prosurvival cytokines is often sufficient for cellular stress-induced cell death through activation of ***caspases***. Although the signaling pathways leading to passive cell death in human and mouse eosinophils have not been compared side by side, they likely rely on similar mechanisms and play a similar role in basal turnover. The causes of actively induced apoptosis vary between human and mouse eosinophils, but there are also many similarities. For example, human[150] and mouse[151] eosinophils undergo apoptosis on ligation of Fas (CD95), particularly in the presence of TNF-α and IFN-γ, leading to activation of caspase-3 and caspase-8. Similarly, human and mouse eosinophils express paralogous sialic acid–binding immunoglobulin-like lectins linked with eosinophil apoptosis (see Table E1). The presence of IL-5 enhances the apoptotic mediating effects of these lectins in human subjects and results in activation of reactive oxygen species and mitochondrial dysfunction[152]; similar effects on mouse eosinophils have yet to be reported.[153] The identification of additional targets, such as CD30[154] and CD300a,[155] described on human eosinophils does not preclude their presence or function on mouse eosinophils. Finally, both human and mouse eosinophils are susceptible to corticosteroid-induced cell death. Corticosteroid binding to receptors on eosinophils is proposed to lead to downregulation of IL-5, inhibition of nuclear factor κB, and the activation of caspases.[156-158] This highlights a key feature for the translational application of mice as a model of human disease: the majority of cellular targets for eosinophil-induced death are retained between these 2 mammalian species.

CONCLUSIONS/SUMMARY

The exhaustive summary presented here and in Tables E1 through E9 provides a clear demonstration of the thesis articulated in the introductory paragraphs. Specifically, genetic, biochemical, molecular, and morphologic differences between human and mouse eosinophils exist. However, most of these differences are often either of unknown significance or "cosmetic" in character, leaving what we believe is an inherent underlying truth: eosinophils and their associated effector functions are activities all mammals gained from a protomammalian ancestor, with specific differences subsequently arising because of selective pressures on each extant mammalian order and, in turn, individual families, species, or both. Thus the structural and, more importantly, functional similarities between human and mouse eosinophils simply reflect the amazing level of propinquity demonstrated from the available genomic sequence studies. This conclusion has significant logistic consequences on our understanding of human disease and the development of therapeutic options. Specifically, the significant overlap and similarities between human and mouse eosinophils suggested by the literature highlights the unique utility of mouse-based studies with an expectation of valid extrapolation to the understanding and treatment of human eosinophil-associated diseases.

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REFERENCES

1. Rosenthal N, Brown S. The mouse ascending: perspectives for human-disease models. Nat Cell Biol 2007;9:993-9.
2. Church DM, Goodstadt L, Hillier LW, Zody MC, Goldstein S, She X, et al. Lineage-specific biology revealed by a finished genome assembly of the mouse. PLoS Biol 2009;7:e1000112.
3. Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, et al. Initial sequencing and comparative analysis of the mouse genome. Nature 2002;420:520-62.
4. Evsikov AV, Dolan ME, Genrich MP, Patek E, Bult CJ. MouseCyc: a curated biochemical pathways database for the laboratory mouse. Genome Biol 2009;10:R84.
5. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. J Immunol 2004;172:2731-8.
6. Kararli TT. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm Drug Dispos 1995;16:351-80.
7. Rangarajan A, Weinberg RA. Opinion: comparative biology of mouse versus human cells: modelling human cancer in mice. Nat Rev Cancer 2003;3:952-9.
8. de Jong M, Maina T. Of mice and humans: are they the same?-implications in cancer translational research. J Nucl Med 2010;51:501-4.
9. Wenzel S, Holgate ST. The mouse trap: it still yields few answers in asthma. Am J Respir Crit Care Med 2006;174:1173-8.
10. Persson CG, Erjefält JS, Korsgren M, Sundler F. The mouse trap. Trends Pharmacol Sci 1997;18:465-7.
11. Wenzel SE. Eosinophils in asthma—closing the loop or opening the door? N Engl J Med 2009;360:1026-8.
12. McKenna MG. The origin and early differentiation of therian mammals. Ann N Y Acad Sci 1969;167:217-40.
13. McGarry MP, Protheroe CA, Lee JJ. Mouse hematology. 1st ed. New York: Cold Spring Harbor Laboratory Press; 2009.
14. McGarry MP. The evolutionary origins and presence of eosinophils in extant species. In: Lee JJ, Rosenberg H, editors. Eosinophils in health and disease. Waltham (MA): Elsevier; 2012.
15. Lee JJ, Jacobsen EA, McGarry MP, Schleimer RP, Lee NA. Eosinophils in health and disease: the *LIAR* hypothesis. Clin Exp Allergy 2010;40:563-75.
16. Iwasaki H, Mizuno S, Mayfield R, Shigematsu H, Arinobu Y, Seed B, et al. Identification of eosinophil lineage-committed progenitors in the murine bone marrow. J Exp Med 2005;201:1891-7.
17. Mori Y, Iwasaki H, Kohno K, Yoshimoto G, Kikushige Y, Okeda A, et al. Identification of the human eosinophil lineage-committed progenitor: revision of phenotypic definition of the human common myeloid progenitor. J Exp Med 2009;206:183-93.
18. Uhm TG, Kim BS, Chung IY. Eosinophil development, regulation of eosinophil-specific genes, and role of eosinophils in the pathogenesis of asthma. Allergy Asthma Immunol Res 2012;4:68-79.
19. McNagny K, Graf T. Making eosinophils through subtle shifts in transcription factor expression. J Exp Med 2002;195:F43-7.

20. Sanderson CJ. Interleukin-5, eosinophils, and disease. Blood 1992;79:3101-9.
21. Clutterbuck EJ, Hirst EM, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GMCSF. Blood 1989;73:1504-12.
22. Dyer KD, Moser JM, Czapiga M, Siegel SJ, Percopo CM, Rosenberg HF. Functionally competent eosinophils differentiated ex vivo in high purity from normal mouse bone marrow. J Immunol 2008;181:4004-9.
23. Radinger M, Lotvall J. Eosinophil progenitors in allergy and asthma—do they matter? Pharmacol Ther 2009;121:174-84.
24. Romanovski DL. On the question of parasitology and therapy of malaria. St Petersburg (Russia): Imperial Medical Military Academy; 1891.
25. Giemsa G. Färbemethoden für malariaparasiten. Zentralbl Bakteriol 1902;31: 429-30.
26. Wright JH. A rapid method for the differential staining of blood films and malarial parasites. J Med Res 1902;7:138-44.
27. Dvorak AM, Ishizaka T. Human eosinophils in vitro. An ultrastructural morphology primer. Histol Histopathol 1994;9:339-74.
28. Miller F, de Harven E, Palade GE. The structure of eosinophil leukocyte granules in rodents and in man. J Cell Biol 1966;31:349-62.
29. Dvorak AM, Furitsu T, Estrella P, Letourneau L, Ishizaka T, Ackerman SJ. Ultrastructural localization of major basic protein in the human eosinophil lineage in vitro. J Histochem Cytochem 1994;42:1443-51.
30. Egesten A, Alumets J, von Mecklenburg C, Palmegren M, Olsson I. Localization of eosinophil cationic protein, major basic protein, and eosinophil peroxidase in human eosinophils by immunoelectron microscopic technique. J Histochem Cytochem 1986;34:1399-403.
31. Denzler KL, Farmer SC, Crosby JR, Borchers MT, Cieslewicz G, Larson KA, et al. Eosinophil major basic protein-1 does not contribute to allergen-induced airway pathologies in mouse models of asthma. J Immunol 2000;165: 5509-17.
32. Denzler KL, Borchers MT, Crosby JR, Cieslewicz G, Hines EM, Justice JP, et al. Extensive eosinophil degranulation and peroxidase-mediated oxidation of airway proteins do not occur in a mouse ovalbumin-challenge model of pulmonary inflammation. J Immunol 2001;167:1672-82.
33. Lee JJ, Lee NA. Eosinophil degranulation: an evolutionary vestige or a universally destructive effector function? Clin Exp Allergy 2005;35:986-94.
34. Melo RC, Spencer LA, Perez SA, Ghiran I, Dvorak AM, Weller PF. Human eosinophils secrete preformed, granule-stored interleukin-4 through distinct vesicular compartments. Traffic 2005;6:1047-57.
35. Spencer LA, Szela CT, Perez SA, Kirchhoffer CL, Neves JS, Radke AL, et al. Human eosinophils constitutively express multiple Th1, Th2, and immunoregulatory cytokines that are secreted rapidly and differentially. J Leukoc Biol 2009;85: 117-23.
36. Wen T, Mingler MK, Blanchard C, Wahl B, Pabst O, Rothenberg ME. The pan-B cell marker CD22 is expressed on gastrointestinal eosinophils and negatively regulates tissue eosinophilia. J Immunol 2012;188:1075-82.
37. Gleich GJ, Adolphson CR, Leiferman KM. The biology of the eosinophilic leukocyte. Ann Rev Med 1993;44:85-101.
38. Ackerman SJ, Liu L, Kwatia MA, Savage MP, Leonidas DD, Swaminathan GJ, et al. Charcot-Leyden crystal protein (galectin-10) is not a dual function galectin with lysophospholipase activity but binds a lysophospholipase inhibitor in a novel structural fashion. J Biol Chem 2002;277:14859-68.
39. Barker RL, Gundel RH, Gleich GJ, Checkel JL, Loegering DA, Pease LR, et al. Acidic polyamino acids inhibit human eosinophil granule major basic protein toxicity. Evidence of a functional role for ProMBP. J Clin Invest 1991;88:798-805.
40. Larson KA, Horton MA, Madden BJ, Gleich GJ, Lee NA, Lee JJ. The identification and cloning of a murine major basic protein gene expressed in eosinophils. J Immunol 1995;155:3002-12.
41. Macias MP, Welch KC, Denzler KL, Larson KA, Lee NA, Lee JJ. The identification of a new murine eosinophil major basic protein (mMBP) gene: cloning and characterization of mMBP-2. J Leukoc Biol 2000;67:567-76.
42. Plager DA, Loegering DA, Weiler DA, Checkel JL, Wagner JM, Clarke NJ, et al. A novel and highly divergent homolog of human eosinophil granule major basic protein. J Biol Chem 1999;274:14464-73.
43. Li MS, Sun L, Satoh T, Fisher LM, Spry CJ. Human eosinophil major basic protein, a mediator of allergic inflammation, is expressed by alternative splicing from two promoters. Biochem J 1995;305:921-7.
44. Ackerman SJ, Kephart GM, Habermann TM, Greipp PR, Gleich GJ. Localization of eosinophil granule major basic protein in human basophils. J Exp Med 1983; 158:946-61.
45. Nakajima T, Matsumoto K, Suto H, Tanaka K, Ebisawa M, Tomita H, et al. Gene expression screening of human mast cells and eosinophils using high-density oligonucleotide probe arrays: abundant expression of major basic protein in mast cells. Blood 2001;98:1127-34.
46. Wagner JM, Hustin J, Bonno M, Kephart GM, Gurian KV, Gleich GJ. Pregnancy-associated major basic protein: deposition of protein and expression of mRNA at the maternal-fetal junction in early and late gestation. Placenta 1994;15:625-40.
47. Rosenberg HF, Ackerman SJ, Tenen DG. Human eosinophil cationic protein. Molecular cloning of a cytotoxin and helminthotoxin with ribonuclease activity. J Exp Med 1989;170:163-76.
48. Rosenberg HF, Tenen DG, Ackerman SJ. Molecular cloning of the human eosinophil-derived neurotoxin: a member of the ribonuclease gene family. Proc Natl Acad Sci U S A 1989;86:4460-4.
49. Cormier SA, Larson KA, Yuan S, Mitchell TL, Lindenberger K, Carrigan P, et al. Mouse Eosinophil-associated ribonucleases: a unique subfamily expressed during hematopoiesis. Mamm Genome 2001;12:352-61.
50. Larson KA, Olson EV, Madden BJ, Gleich GJ, Lee NA, Lee JJ. Two highly homologous ribonuclease genes expressed in mouse eosinophils identify a larger subgroup of the mammalian ribonuclease superfamily. Proc Natl Acad Sci U S A 1996;93:12370-5.
51. Rosenberg HF, Dyer KD, Tiffany HL, Gonzalez M. Rapid evolution of a unique family of primate ribonuclease genes. Nat Genet 1995;10:219-23.
52. Cormier SA, Yuan S, Crosby JR, Protheroe CA, Dimina DM, Hines EM, et al. T(H)2-mediated pulmonary inflammation leads to the differential expression of ribonuclease genes by alveolar macrophages. Am J Respir Cell Mol Biol 2002; 27:678-87.
53. Sur S, Glitz DG, Kita H, Kujawa SM, Peterson EA, Weiler DA, et al. Localization of eosinophil-derived neurotoxin and eosinophil cationic protein in neutrophilic leukocytes. J Leukoc Biol 1998;63:715-22.
54. Shapiro R, Vallee BL. Interaction of human placental ribonuclease with placental ribonuclease inhibitor. Biochemistry 1991;30:2246-55.
55. Horton MA, Larson KA, Lee JJ, Lee NA. Cloning of the murine eosinophil peroxidase gene (mEPO): characterization of a conserved subgroup of mammalian hematopoietic peroxidases. J Leukoc Biol 1996;60:285-94.
56. Ten RM, Pease LR, McKean DJ, Bell MP, Gleich GJ. Molecular cloning of the human eosinophil peroxidase. J Exp Med 1989;169:1757-69.
57. Weller PF, Goetzl EJ, Austen KF. Identification of human eosinophil lysophospholipase as the constituent of Charcot-Leyden crystals. Proc Natl Acad Sci U S A 1980;77:7440-3.
58. Kubach J, Lutter P, Bopp T, Stoll S, Becker C, Huter E, et al. Human CD4+CD25+ regulatory T cells: proteome analysis identifies galectin-10 as a novel marker essential for their anergy and suppressive function. Blood 2007; 110:1550-8.
59. Erlich P. Ueber die Specifischen granulationen des Blutes [in German]. Arch Anant Physiol 1879;3:571-9.
60. Huber HL, Koessler KK. The pathology of bronchial asthma. Arch Intern Med 1922;30:689-760.
61. Lee JJ, Rosenberg H, editors. Eosinophils in health and disease. Waltham (MA): Elsevier; 2012.
62. Jacobsen EA, Lee JJ, Lee NA. Eosinophils in health and disease … did you know? Blood. In press 2012.
63. Borchers MT, Ansay TL, DeSalle R, Daugherty BL, Shen HH, Metzger M, et al. In vitro assessment of chemokine receptor-ligand interactions mediating mouse eosinophil migration. J Leukoc Biol 2002;71:1033-41.
64. Rot A, Krieger M, Brunner T, Bischoff SC, Schall TJ, Dahinden CA. RANTES and macrophage inflammatory protein 1 alpha induce the migration and activation of normal human eosinophil granulocytes. J Exp Med 1992;176:1489-95.
65. Das AM, Ajuebor MN, Flower RJ, Perretti M, McColl SR. Contrasting roles for RANTES and macrophage inflammatory protein-1 alpha (MIP-1 alpha) in a murine model of allergic peritonitis. Clin Exp Immunol 1999;117:223-9.
66. Gonzalo JA, Lloyd CM, Wen D, Albar JP, Wells TN, Proudfoot A, et al. The coordinated action of CC chemokines in the lung orchestrates allergic inflammation and airway hyperresponsiveness. J Exp Med 1998;188:157-67.
67. Lee JB, Matsumoto T, Shin YO, Yang HM, Min YK, Timothy O, et al. The role of RANTES in a murine model of food allergy. Immunol Invest 2004;33:27-38.
68. Schall TJ, Simpson NJ, Mak JY. Molecular cloning and expression of the murine RANTES cytokine: structural and functional conservation between mouse and man. Eur J Immunol 1992;22:1477-81.
69. Jampilek J, Dolezal M, Opletalova V, Hartl J. 5-Lipoxygenase, leukotrienes biosynthesis and potential antileukotrienic agents. Curr Med Chem 2006;13:117-29.
70. Grant GE, Rokach J, Powell WS. 5-Oxo-ETE and the OXE receptor. Prostaglandins Other Lipid Mediat 2009;89:98-104.
71. Jones CE, Holden S, Tenaillon L, Bhatia U, Seuwen K, Tranter P, et al. Expression and characterization of a 5-oxo-6E,8Z,11Z,14Z-eicosatetraenoic acid receptor highly expressed on human eosinophils and neutrophils. Mol Pharmacol 2003; 63:471-7.
72. Powell WS, Chung D, Gravel S. 5-oxo-6,8,11,14-eicosatetraenoic acid is a potent stimulator of human eosinophil migration. J Immunol 1995;154:4123-32.

73. Powell WS, Rokach J. Biochemistry, biology and chemistry of the 5-lipoxygenase product 5-oxo-ETE. Prog Lipid Res 2005;44:154-83.
74. Hevko JM, Bowers RC, Murphy RC. Synthesis of 5-oxo-6,8,11,14-eicosatetraenoic acid and identification of novel omega-oxidized metabolites in the mouse macrophage. J Pharmacol Exp Ther 2001;296:293-305.
75. Lacy P, Moqbel R. Immune effector functions of eosinophils in allergic airway inflammation. Curr Opin Allergy Clin Immunol 2001;1:79-84.
76. Lacy P, Moqbel R. Eosinophil cytokines. Chem Immunol 2000;76:134-55.
77. Logan MR, Lacy P, Bablitz B, Moqbel R. Expression of eosinophil target SNAREs as potential cognate receptors for vesicle-associated membrane protein-2 in exocytosis. J Allergy Clin Immunol 2002;109:299-306.
78. Logan MR, Lacy P, Odemuyiwa SO, Steward M, Davoine F, Kita H, et al. A critical role for vesicle-associated membrane protein-7 in exocytosis from human eosinophils and neutrophils. Allergy 2006;61:777-84.
79. Moqbel R, Lacy P. Molecular mechanisms in eosinophil activation. Chem Immunol 2000;78:189-98.
80. Stanley AC, Lacy P. Pathways for cytokine secretion. Physiology (Bethesda) 2010;25:218-29.
81. Abu-Ghazaleh RI, Fujisawa T, Mestecky J, Kyle RA, Gleich GJ. IgA-induced eosinophil degranulation. J Immunol 1989;142:2393-400.
82. Ochkur SI, Kim JD, Protheroe CA, Colbert D, Moqbel R, Lacy P, et al. The development of a sensitive and specific ELISA for mouse eosinophil peroxidase: assessment of eosinophil degranulation ex vivo and in models of human disease. J Immunol Methods 2012;375:138-47.
83. Shamri R, Melo RC, Young KM, Bivas-Benita M, Xenakis JJ, Spencer LA, et al. CCL11 elicits secretion of RNases from mouse eosinophils and their cell-free granules. FASEB J 2012;26:2084-93.
84. Mould AW, Ramsay AJ, Matthaei KI, Young IG, Rothenberg ME, Foster PS. The effect of IL-5 and eotaxin expression in the lung on eosinophil trafficking and degranulation and the induction of bronchial hyperreactivity. J Immunol 2000;164:2142-50.
85. Clark K, Simson L, Newcombe N, Koskinen AM, Mattes J, Lee NA, et al. Eosinophil degranulation in the allergic lung of mice primarily occurs in the airway lumen. J Leukoc Biol 2004;75:1001-9.
86. Ochkur SI, Jacobsen EA, Protheroe CA, Biechele TL, Pero RS, McGarry MP, et al. Co-expression of IL-5 and eotaxin-2 in mice creates an eosinophil-dependent model of respiratory inflammation with characteristics of severe asthma. J Immunol 2007;178:7879-89.
87. Erjefalt JS, Persson CG. New aspects of degranulation and fates of airway mucosal eosinophils. Am J Respir Crit Care Med 2000;161:2074-85.
88. Greiff L, Erjefält JS, Andersson M, Svensson C, Persson CG. Generation of clusters of free eosinophil granules (Cfegs) in seasonal allergic rhinitis. Allergy 1998;53:200-3.
89. Leiferman KM. Eosinophils in atopic dermatitis. Allergy 1989;44(suppl 9):20-6.
90. Willetts L, Parker K, Wesselius LJ, Protheroe CA, Jaben E, Graziano P, et al. Immunodetection of occult eosinophils in lung tissue biopsies may help predict survival in acute lung injury. Respir Res 2011;12:116.
91. Protheroe CA, Woodruff SA, DePetris G, Mukkada V, Ochkur SI, Janarthanan S, et al. A novel histological scoring system to evaluate mucosal biopsies from patients with eosinophilic esophagitis. Clin Gastroenterol Hepatol 2009;7:749-55.
92. Aceves SS, Newbury RO, Dohil R, Bastian JF, Broide DH. Esophageal remodeling in pediatric eosinophilic esophagitis. J Allergy Clin Immunol 2007;119:206-12.
93. Persson CG, Erjefalt JS. "Ultimate activation" of eosinophils in vivo: lysis and release of clusters of free eosinophil granules (Cfegs). Thorax 1997;52:569-74.
94. Neves JS, Perez SA, Spencer LA, Melo RC, Reynolds L, Ghiran I, et al. Eosinophil granules function extracellularly as receptor-mediated secretory organelles. Proc Natl Acad Sci U S A 2008;105:18478-83.
95. Hogan SP, Mishra A, Brandt EB, Royalty MP, Pope SM, Zimmermann N, et al. A pathological function for eotaxin and eosinophils in eosinophilic gastrointestinal inflammation. Nat Immunol 2001;2:353-60.
96. Erjefält JS, Andersson M, Greiff L, Korsgren M, Gizycki M, Jeffery PK, et al. Cytolysis and piecemeal degranulation as distinct modes of activation of airway mucosal eosinophils. J Allergy Clin Immunol 1998;102:286-94.
97. Filley WV, Holley KE, Kephart GM, Gleich GJ. Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. Lancet 1982;2:11-6.
98. Bink K, Walch A, Feuchtinger A, Eisenmann H, Hutzler P, Hofler H, et al. TO-PRO-3 is an optimal fluorescent dye for nuclear counterstaining in dual-colour FISH on paraffin sections. Histochem Cell Biol 2001;115:293-9.
99. Malm-Erjefält M, Persson CGA, Erjefalt JS. Degranulation status of airway tissue eosinophils in mouse models of allergic airway inflammation. Am J Respir Cell Mol Biol 2001;24:352-9.
100. Stelts D, Egan RW, Falcone A, Garlisi CG, Gleich GJ, Kreutner W, et al. Eosinophils retain their granule major basic protein in a murine model of allergic pulmonary inflammation. Am J Respir Cell Mol Biol 1998;18:9463-70.
101. Rothenberg ME, Hogan SP. The eosinophil. Annu Rev Immunol 2006;24:147-74.
102. Ackerman SJ, Bochner BS. Mechanisms of eosinophilia in the pathogenesis of hypereosinophilic disorders. Immunol Allergy Clin North Am 2007;27:357-75.
103. Blanchard C, Rothenberg ME. Biology of the eosinophil. Adv Immunol 2009;101:81-121.
104. Kita H. Eosinophils: multifaceted biological properties and roles in health and disease. Immunol Rev 2011;242:161-77.
105. Spencer L, Weller P. Eosinophils and Th2 immunity: contemporary insights. Immunol Cell Biol 2010;88:250-6.
106. Dyer KD, Percopo CM, Xie Z, Yang Z, Kim JD, Davoine F, et al. Mouse and human eosinophils degranulate in response to platelet-activating factor (PAF) and lysoPAF via a PAF-receptor-independent mechanism: evidence for a novel receptor. J Immunol 2010;184:6327-34.
107. Shamri R, Xenakis JJ, Spencer LA. Eosinophils in innate immunity: an evolving story. Cell Tissue Res 2011;343:57-83.
108. Akuthota P, Wang HB, Spencer LA, Weller PF. Immunoregulatory roles of eosinophils: a new look at a familiar cell. Clin Exp Allergy 2008;38:1254-63.
109. Moqbel R, Levi-Schaffer F, Kay AB. Cytokine generation by eosinophils. J Allergy Clin Immunol 1994;94:1183-8.
110. Ohno I, Ohtani H, Nitta Y, Suzuki J, Hoshi H, Honma M, et al. Eosinophils as a source of matrix metalloproteinase-9 in asthmatic airway inflammation. Am J Respir Cell Mol Biol 1997;16:212-9.
111. Tseng YK, Tu WC, Lee HH, Chen KM, Chou HL, Lai SC. Ultrastructural localization of matrix metalloproteinase-9 in eosinophils from the cerebrospinal fluid of mice with eosinophilic meningitis caused by Angiostrongylus cantonensis. Ann Trop Med Parasitol 2004;98:831-41.
112. Kanaoka Y, Boyce JA. Cysteinyl leukotrienes and their receptors: cellular distribution and function in immune and inflammatory responses. J Immunol 2004;173:1503-10.
113. Christie PE, Henderson WR Jr. Lipid inflammatory mediators: leukotrienes, prostaglandins, platelet-activating factor. Clin Allergy Immunol 2002;16:233-54.
114. Wong CK, Cheung PF, Ip WK, Lam CW. Intracellular signaling mechanisms regulating toll-like receptor-mediated activation of eosinophils. Am J Respir Cell Mol Biol 2007;37:85-96.
115. Takeda K, Akira S. Toll-like receptors in innate immunity. Int Immunol 2005;17:1-14.
116. Matsuwaki Y, Wada K, Moriyama H, Kita H. Human eosinophil innate response to Alternaria fungus through protease-activated receptor-2. Int Arch Allergy Immunol 2011;155(suppl 1):123-8.
117. Schmidlin F, Amadesi S, Dabbagh K, Lewis DE, Knott P, Bunnett NW, et al. Protease-activated receptor 2 mediates eosinophil infiltration and hyperreactivity in allergic inflammation of the airway. J Immunol 2002;169:5315-21.
118. Coughlin SR. Thrombin signalling and protease-activated receptors. Nature 2000;407:258-64.
119. Giembycz MA, Lindsay MA. Pharmacology of the eosinophil. Pharmacol Rev 1999;51:213-340.
120. Yousefi S, Gold JA, Andina N, Lee JJ, Kelly AM, Kozlowski E, et al. Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense. Nat Med 2008;14:949-53.
121. Weller PF, Rand TH, Barrett T, Elovic A, Wong DT, Finberg RW. Accessory cell function of human eosinophils. HLA-DR-dependent, MHC-restricted antigen-presentation and IL-1 alpha expression. J Immunol 1993;150:2554-62.
122. Lucey DR, Nicholson-Weller A, Weller PF. Mature human eosinophils have the capacity to express HLA-DR. Proc Natl Acad Sci U S A 1989;86:1348-51.
123. Akuthota P, Wang H, Weller PF. Eosinophils as antigen-presenting cells in allergic upper airway disease. Curr Opin Allergy Clin Immunol 2010;10:14-9.
124. Shi HZ, Humbles A, Gerard C, Jin Z, Weller PF. Lymph node trafficking and antigen presentation by endobronchial eosinophils. J Clin Invest 2000;105:945-53.
125. Padigel UM, Lee JJ, Nolan TJ, Schad GA, Abraham D. Eosinophils can function as antigen-presenting cells to induce primary and secondary immune responses to *Strongyloides stercoralis*. Infect Immun 2006;74:3232-8.
126. Wang H-B, Ghiran I, Matthaei K, Weller PF. Airway eosinophils: allergic inflammation recruited professional antigen-presenting cells. J Immunol 2007;179:7585-92.
127. Shi H-Z, Xiao C-Q, Li C-Q, Mo X-Y, Yang Q-L, Leng J, et al. Endobronchial eosinophils preferentially stimulate T helper cell type 2 responses. Allergy 2004;59:428-35.
128. MacKenzie JR, Mattes J, Dent LA, Foster PS. Eosinophils promote allergic disease of the lung by regulating $CD4^+$ Th2 lymphocyte function. J Immunol 2001;167:3146-55.

129. Jacobsen EA, Zellner KR, Colbert D, Lee NA, Lee JJ. Eosinophils regulate dendritic cells and Th2 pulmonary immune responses following allergen provocation. J Immunol 2011;187:6059-68.
130. Percopo CM, Qiu Z, Phipps S, Foster PS, Domachowske JB, Rosenberg HF. Pulmonary eosinophils and their role in immunopathologic responses to formalin-inactivated pneumonia virus of mice. J Immunol 2009;183:604-12.
131. Goldman M, Le Moine A, Braun M, Flamand V, Abramowicz D. A role for eosinophils in transplant rejection. Trends Immunol 2001;22:247-51.
132. Correale J, Fiol M. Activation of humoral immunity and eosinophils in neuromyelitis optica. Neurology 2004;63:2363-70.
133. Masterson JC, Furuta GT, Lee JJ. Update on clinical and immunological features of eosinophilic gastrointestinal diseases. Curr Opin Gastroenterol 2011;27: 515-22.
134. Chu VT, Frohlich A, Steinhauser G, Scheel T, Roch T, Fillatreau S, et al. Eosinophils are required for the maintenance of plasma cells in the bone marrow. Nat Immunol 2011;12:151-9.
135. Tokoyoda K, Hauser AE, Nakayama T, Radbruch A. Organization of immunological memory by bone marrow stroma. Nat Rev Immunol 2010;10:193-200.
136. Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science 2011;332:243-7.
137. Chawla A, Nguyen KD, Goh YP. Macrophage-mediated inflammation in metabolic disease. Nat Rev Immunol 2011;11:738-49.
138. Gouon-Evans V, Rothenberg ME, Pollard JW. Postnatal mammary gland development requires macrophages and eosinophils. Development 2000;127: 2269-82.
139. Timmons BC, Fairhurst AM, Mahendroo MS. Temporal changes in myeloid cells in the cervix during pregnancy and parturition. J Immunol 2009;182:2700-7.
140. Foster EL, Simpson EL, Fredrikson LJ, Lee JJ, Lee NA, Fryer AD, et al. Eosinophils increase neuron branching in human and murine skin and in vitro. PLoS One 2011;6:e22029.
141. Raap U, Wardlaw AJ. A new paradigm of eosinophil granulocytes: neuroimmune interactions. Exp Dermatol 2008;17:731-8.
142. Throsby M, Herbelin A, Pleau JM, Dardenne M. CD11c+ eosinophils in the murine thymus: developmental regulation and recruitment upon MHC class I-restricted thymocyte deletion. J Immunol 2000;165:1965-75.
143. Kim HJ, Alonzo ES, Dorothee G, Pollard JW, Sant'Angelo DB. Selective depletion of eosinophils or neutrophils in mice impacts the efficiency of apoptotic cell clearance in the thymus. PLoS One 2010;5:e11439.
144. Tulic MK, Sly PD, Andrews D, Crook M, Davoine F, Odemuyiwa SO, et al. Thymic indoleamine 2,3-dioxygenase-positive eosinophils in young children: potential role in maturation of the naive immune system. Am J Pathol 2009; 175:2043-52.
145. Nagase H, Okugawa S, Ota Y, Yamaguchi M, Tomizawa H, Matsushima K, et al. Expression and function of Toll-like receptors in eosinophils: activation by Toll-like receptor 7 ligand. J Immunol 2003;171:3977-82.
146. Kvarnhammar AM, Cardell LO. Pattern-recognition receptors in human eosinophils. Immunology 2012;136:11-20.
147. Phipps S, Lam CE, Mahalingam S, Newhouse M, Ramirez R, Rosenberg HF, et al. Eosinophils contribute to innate antiviral immunity and promote clearance of respiratory syncytial virus. Blood 2007;110:1578-86.
148. Yousefi S, Hoessli DC, Blaser K, Mills GB, Simon HU. Requirement of Lyn and Syk tyrosine kinases for the prevention of apoptosis by cytokines in human eosinophils. J Exp Med 1996;183:1407-14.
149. Geijsen N, Koenderman L, Coffer PJ. Specificity in cytokine signal transduction: lessons learned from the IL-3/IL-5/GM-CSF receptor family. Cytokine Growth Factor Rev 2001;12:19-25.
150. Letuve S, Druilhe A, Grandsaigne M, Aubier M, Pretolani M. Involvement of caspases and of mitochondria in Fas ligation-induced eosinophil apoptosis: modulation by interleukin-5 and interferon-gamma. J Leukoc Biol 2001;70:767-75.
151. Tong J, Bandulwala HS, Clay BS, Anders RA, Shilling RA, Balachandran DD, et al. Fas-positive T cells regulate the resolution of airway inflammation in a murine model of asthma. J Exp Med 2006;203:1173-84.
152. Nutku E, Aizawa H, Hudson SA, Bochner BS. Ligation of Siglec-8: a selective mechanism for induction of human eosinophil apoptosis. Blood 2003;101:5014-20.
153. Zimmermann N, McBride ML, Yamada Y, Hudson SA, Jones C, Cromie KD, et al. Siglec-F antibody administration to mice selectively reduces blood and tissue eosinophils. Allergy 2008;63:1156-63.
154. Berro AI, Perry GA, Agrawal DK. Increased expression and activation of CD30 induce apoptosis in human blood eosinophils. J Immunol 2004;173:2174-83.
155. Munitz A, Bachelet I, Eliashar R, Moretta A, Moretta L, Levi-Schaffer F. The inhibitory receptor IRp60 (CD300a) suppresses the effects of IL-5, GM-CSF, and eotaxin on human peripheral blood eosinophils. Blood 2006;107:1996-2003.
156. Chauhan S, Leach CH, Kunz S, Bloom JW, Miesfeld RL. Glucocorticoid regulation of human eosinophil gene expression. J Steroid Biochem Mol Biol 2003;84: 441-52.
157. Walsh GM, Sexton DW, Blaylock MG. Corticosteroids, eosinophils and bronchial epithelial cells: new insights into the resolution of inflammation in asthma. J Endocrinol 2003;178:37-43.
158. Bloom JW, Chacko J, Lohman IC, Halonen M, Martinez FD, Miesfeld RL. Differential control of eosinophil survival by glucocorticoids. Apoptosis 2004;9: 97-104.