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Eosinophils from A to Z

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Abstract

Eosinophils are bone marrow-derived granulocytes and are found in low numbers in the peripheral blood of healthy subjects. In type 2 inflammatory diseases, eosinopoiesis in the bone marrow is increased, resulting in a rise in the number of mature eosinophils released in the circulation. From the blood, eosinophils can migrate in multiple tissues and organs under both physiological and pathological conditions. Eosinophils exert their various functions through the synthesis and release of a variety of granule proteins and pro-inflammatory mediators. Despite being present in all species of vertebrates, the functional role of eosinophils is still a matter of debate. Eosinophils may play a role in host defense against various pathogens. In addition, eosinophils have been reported to be involved in tissue homeostasis and exhibit immunomodulatory activities. In this review, we aim to provide a broad overview of eosinophil biology and eosinophilic diseases in a lexicon-style format using keywords starting from A until Z with crossreferences to other chapters indicated in italic in the text or specified in parentheses.

Keywords: eosinophil, eosinopoiesis, eosinophil subsets, immunoregulation, tissue homeostasis

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Activation

Blood eosinophils are found in either non-activated, pre-activated ("primed") or fully activated states[1,2]. Non-activated eosinophils are characterized by low surface expression of CD69, a

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surrogate marker for eosinophil activation, and expression of integrins in a non-activated state[3]. If eosinophils are primed through *cytokines* such as interleukin (IL)-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) prior to their activation, they exhibit increased susceptibility towards stimulating factors[4,5]. These factors include *cytokines* and inflammatory mediators such as IL-3, IL-4, IL-5, IL-13, GM-CSF, C-C motif chemokine ligand (CCL)2, CCL3, CCL5, CCL11, histamine, adenosine, LL-37, leukotriene C_4 (LTC_4), and leukotriene D_4 (LTD_4) that are released by immune cells, epithelial cells, fat cells, fibroblasts, and endothelial cells, as well as pathogens[6-12] (Table 1). Activation of circulating eosinophils induces their arrest on the activated endothelium, tissue transmigration[1], modulates their function through the secretion of preformed mediators stored in the *granules* or newly synthesized molecules[13] (see *"Degranulation"*), superoxide production[4], enhanced leukotriene synthesis[4], and prolong their survival[4]. Additionally, activation of human eosinophils was shown to be characterized by changes in the expression pattern of a variety of proteins and phosphoproteins[3] that are thought to be involved in eosinophil survival (see *"Apoptosis"*) and migration by the activation of adhesion molecules and cell surface proteins (see *"Trafficking"*)[1,3], as well as by distinct expression pattern depending on the stimulating cytokine[3].

Allergy

Allergy, originally described as specifically altered reactivity of the organism, defines an immunologically mediated, allergen-specific hypersensitivity that can affect most organs. Even though four types (I, immunoglobulin (Ig)E-mediated; II, cytotoxic; III, immune complex; IV, delayed-type hypersensitivity) have been distinguished, there seems to be a continuum, e.g. as certain drugs can cause different types of allergic reactions, even in one patient, and combinations of type I and IV reactions as seen in atopic dermatitis may occur[14] (see "*Atopic diseases*").

Based on the immune mechanisms, eosinophils are mainly involved in late-phase responses of IgE-mediated and T cell-mediated hypersensitivity reactions (Table 2). Tissue and blood eosinophilia are often used as criteria for differential diagnosis of allergic diseases[14,15] (see *"Biomarkers"*, *"Normal levels"*).

Eosinophils promote allergic inflammation through the release of pro-inflammatory mediators such as LTC_4, major basic protein (*MBP*), eosinophil cationic protein (*ECP*), IL-4, and IL-13 (see *"Cytokines"*)[4]. Hence, circulating levels of eosinophil mediators including ECP, eosinophil-derived neurotoxin (*EDN*), and eosinophil peroxidase (*EPX*), as well as eosinophilia-associated serum markers (e.g. soluble IL-2 receptors, IL-5, GM-CSF, and soluble sialic acid-binding immunoglobulin-like lectin (siglec)-8) are found in the blood of allergic patients correlating with disease severity[4,16,17] (see *"Biomarkers"*). Eosinophil-mast cell (MC) interactions (see *"Immunoregulation"*, *"Apoptosis"*, "*Activation")* play a pathogenic role in several allergic diseases[18]. Probably, a dysregulated fatty acid *metabolism* in eosinophils contributes to persistent inflammation and progression of allergic diseases[19].

Apoptosis

Circulating eosinophils experience a short half-life of around 8-18 hours[20], in contrast to their prolonged life span of up to 14 days in tissues[21,22] (see *"Kinetics"*). The absence of external survival-prolonging stimuli such as GM-CSF, IL-5, or IL-3 (see *"Cytokines"*) leads to the induction of programmed spontaneous apoptosis within a few days[23,24]. Circulating human

eosinophils were demonstrated to be redistributed to the liver, as well as the spleen and the bone marrow for their elimination[24,25]. Tissue eosinophils have no ability to leave the tissue and reenter the circulation and have therefore to be eliminated *in situ* upon completion of their *function*[26]. Elimination of eosinophils in both scenarios is dependent on tightly regulated apoptosis and subsequent efferocytosis by professional phagocytes like macrophages[27], as well as non-professional phagocytes such as dendritic cells (DCs), lung fibroblasts, and epithelial cells[28].

Eosinophil apoptosis is mediated by the activation of caspase cascades[24]:

- Caspase 9: initiator caspase in response to mitochondrial apoptotic pathway, processed during spontaneous and induced apoptosis
- Caspase 8: critical initiator caspase in Fas ligand (FasL)-mediated apoptosis
- Caspases 3 and 6: executioner caspases involved in spontaneous and induced apoptosis

Furthermore, death receptors of the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor superfamily contribute to the regulation of apoptosis[29]. Additionally, Fas ligation, ligation of TNF receptor (TNFR) family member CD30, and pharmacological agents such as glucocorticoids accelerate eosinophil apoptosis[24].

Failure in apoptotic clearance or excessive eosinophil recruitment are frequently observed in chronic inflammatory diseases[30]. Consequently, therapies targeting eosinophil apoptosis represent a relevant strategy in the *treatment* of eosinophilic diseases[31,32].

Atopic diseases

Atopic diseases comprise atopic dermatitis (AD), bronchial asthma, allergic rhinitis and/or conjunctivitis (i.e. hay fever), and eosinophilic esophagitis[33]. Atopy describes a genetic disposition to develop excessive immune responses to environmental allergens such as aeroallergens and food allergens. Although the term atopy is often equated to high IgE levels, it became clear that in addition to IgE-mediated reactions, e.g. in allergic rhinitis, other factors, in particular barrier defects and T helper 2 immune reactions, account for the pathogenesis of atopic diseases that often follow a chronic disease course[34] (Table 2). Increased levels of circulating eosinophils and *granule* content release in the target tissue are typical features of atopic diseases[35] (see *"Biomarkers"*). Moreover, eosinophilic inflammation in atopic diseases has recently been shown to be mediated by thymic stronal lymphopoietin (TSLP), IL-25, IL-33[36] (see *"Cytokines"*). Furthermore, the phenomenon of atopic march describes the sequential development of atopic diseases in childhood, starting with AD and food allergy, followed by allergic asthma, allergic rhinitis, and eosinophilic esophagitis[33,37,38].

Autophagy

Autophagy is an intracellular conserved catabolic process responsible for the turnover of organelles and macromolecules that will ultimately be degraded by lysosomal hydrolytic enzymes[39]. It is regulated by distinct autophagy-related (ATG) proteins and can be induced by amino acid starvation, lack of energy, and the inhibition of the mammalian target of rapamycin complex 1 (mTORC1)[39]. Interestingly, impairment of autophagy was shown to contribute to different eosinophilic inflammatory diseases such as asthma, Crohn's disease, bronchial asthma, eosinophilic esophagitis (EoE), and chronic rhinosinusitis, as well as

infectious, inflammatory, and autoimmune skin diseases[40,41]. This process has been demonstrated to be crucial for the functionality of immune cells, including eosinophils[39,40].

A reduction of proliferation, maturation, and in the number of mature eosinophils in the circulation and peripheral tissues were observed in human and mouse eosinophils lacking *ATG5/Atg5*[42]. Surprisingly, the absence of *ATG5/Atg5* was shown to increase eosinophil effector *functions* such as *degranulation*, eosinophil extracellular trap (*EET*) formation, and bacterial clearance (see *"Host defense"*)[43]. Moreover, a recent study reported a protective effect of autophagy against cytolysis of mature eosinophils in humans[44] (see *"Degranulation"*).

Autoimmune diseases

There is growing evidence that eosinophils are involved in autoimmune diseases such as bullous pemphigoid (BP), inflammatory bowel diseases (IBD), eosinophilic granulomatosis with polyangiitis (EGPA), eosinophilic myocarditis (EM), neuromyelitis optica (NMO), and primary biliary cirrhosis among others[41,45,46] (Table 2). In autoimmune diseases, eosinophils are found to cause tissue and cell damage through the release of granule proteins (see *"Degranulation"*) or by the process of antibody-dependent cellular cytotoxicity (ADCC)[46]. Furthermore, eosinophils modulate adaptive immune responses (see *"Immunoregulation"*) by activating T cells due to their antigen-presenting capability, affecting T cell differentiation into T helper (Th)2 cells and promoting B cell responses[46]. On the other hand, eosinophils are known to mediate protective functions by contributing to tissue remodeling, tissue repair, angiogenesis, and fibrosis through the release of *growth factors*[46] (see *"Wound healing & tissue remodeling"*).

Biomarkers

Biomarkers are defined as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention"[47]. Distinct biomarkers including the number of circulating eosinophils, organ-specific eosinophil levels, serum levels of eosinophil granule proteins, and fractional exhaled nitric oxide (FeNO) are used for the determination of different eosinophil-associated diseases such as *hypereosinophilic syndrome* (HES), asthma, EGPA and EoE among others[48] (Table 2).

- Blood or tissue eosinophil numbers are the most commonly used biomarkers in clinical practice[49] (see *"Normal levels"*). Blood eosinophil count can be an indicator of disease activity in *HES* patients[49] and act as a biomarker for chronic obstructive pulmonary disease (COPD)[50], and severe eosinophilic asthma[51].
- *ECP* is found in the sputum, saliva, serum, plasma, and broncho-alveolar lavage fluids[52,53]. ECP is the most widely used marker for eosinophil activity and turnover in serum and plasma[54]. It is used as a biomarker for asthma and eosinophilic airway inflammation[52,53], drug eruption[55], psoriasis[55], acute urticaria[55], BP[56], coronary artherosclerosis[57], and outcome of melanoma patients[58]. It is important to note that asthmatic patients with a smoking history have a significant increase in sputum ECP levels[53].
- *EDN* can be measured in serum, sputum, bronchoalveolar lavage fluid, nasal lavage fluid, and urine[59,60]. EDN contributes as a biomarker to the diagnosis of asthma and allergic rhinitis, as well as to assess the severity of the diseases[59,61,62]. For instance, increased

EDN levels are associated with respiratory syncytial virus (RSV) infection, asthma, chronic rhinosinusitis, and *HES*[49,50,63]. In contrast to ECP, EDN has been proposed to be a more accurate biomarker as its levels are not affected by smoking or circadian rhythm[62]. Moreover, EDN in urine has been proposed as a biomarker for asthma and AD, as well as in patients with *HES* and EGPA[54,60]. Furthermore, the measurement of biomarkers such as EDN in the urine is proposed as a non-invasive method and is unaffected by cellular lysis[60].

- Galectin-10 (Gal-10), also known as *Charcot-Leyden Crystal (CLC) protein*, is found in the sputum, nasal secretions, nasal polyps, serum, and skin tissue[64]. The presence of Gal-10 at the above-mentioned sites is indicative of asthma, EoE, rhinitis, sinusitis, AD, and EGPA in regard to diagnosis, disease activity, and treatment effectiveness[64].
- Soluble siglec-8 was identified as a new potential serum marker for asthma, *HES*, and lymphocytic variant of *HES*[16,17,65].
- FeNO is used as a biomarker for asthma[66,67]. Moreover, in combination with a high blood eosinophil count (see *"Normal levels"*), it may identify asthmatic patients with an increased risk of severe exacerbation[67].
- Soluble CD48 measured in the serum was recently proposed as a biomarker for non-allergic asthma[68].

Cancer

Eosinophils are frequently present in tumor microenvironments (TME) and are recruited by growth factors produced by tumor cells, such as IL-5[69] (see *"Trafficking"*). The infiltration of eosinophils into TME can lead to tumor-associated tissue eosinophilia (TATE)[70] – a condition that was demonstrated to be crucial for the control of tumor growth in colorectal cancer (CRC) models[71]. Eosinophils were reported to exert an anti-tumoral role via *degranulation* in several tumors including colon cancer, melanoma, lung cancer, and oral squamous cell carcinoma[70] (Figure 1). They are known to promote $CD8^+$ T cell recruitment into the TME indirectly through the secretion of *chemokines* and to promote macrophage polarization toward the pro-inflammatory (M1) phenotype[69] (see *"Immunoregulation"*). Moreover, GM-CSF-induced activation of transcription factor interferon regulatory factor (IRF)-5 promotes eosinophil-driven type 1 T cell response against tumors, which is counterregulated by IL-10[71]. Direct contact of human eosinophils and natural killer (NK) cells results in the upregulation of the effector functions of NK cells leading to an increase in interferon (IFN)-γ production, cytotoxic activity against tumor cells, and DCs editing process[72]. Moreover, this interaction induces eosinophil *activation*, as well as enhances their antigen-presenting capability[72]. Type 2 innate lymphoid cells (ILC2s) in the TME were shown to interact with eosinophils through the release of type 2 cytokines (IL-4, IL-5, and IL-13) that directly affect eosinophil proliferation and indirectly mediate eosinophil recruitment into the TME via macrophage and epithelial cell-derived eotaxins[73].

It is important to mention, that peripheral blood and tissue eosinophils in TME have been linked to both favorable and bad outcomes[69]. Indeed, mouse eosinophils were also shown to facilitate metastatic niches[69], enhance tumor cell migration and metastatic growth[74], and promote the suppressive phenotype of tumor-associated macrophages suggesting pro-tumorigenic activities[69] (Figure 1). Moreover, the occurrence of TATE has been linked with the local recurrence of urothelial carcinoma[75].

Currently, the use of blood eosinophil count as a potential predictive factor for the outcome of immunotherapy is under discussion. Increased eosinophil numbers correlated with positive response to immune checkpoint treatment, but also increased risk of adverse effects in patients with melanoma, renal cell carcinoma and non-small lung cancer[76-79]. Conversely, higher blood eosinophil count was associated with the recurrence of bladder cancer in patients that received Bacillus Calmette-Guérin (BCG) maintenance therapy[80].

Charcot-Leyden crystal protein

CLC/Gal-10 is one of the most abundant proteins in peripheral blood eosinophils[81] (Table 3A). It should be noted that only eosinophils from human and a few non-human primates exhibit CLC/Gal-10 expression[81]. CLC/Gal-10 was initially believed to be eosinophil specific. It was later shown to be expressed in basophils[82] and, more recently, in regulatory T cells (Treg), although its function in Tregs remains controversial[83]. The elongated hexagonal bipyramidal structure of CLC is formed through clustering of Gal-10 subsequently to eosinophil *degranulation*[84]. The presence of CLC was demonstrated in a small fraction of eosinophil "primary" *granules*[85,86], the nucleus including nuclear matrix[87,88], extra-organellar cytoplasm[87], and at the plasma membrane of mature eosinophils[89]. It is worth mentioning that, unlike other granule proteins, the release of CLC is not mediated through piecemeal degranulation (PMD) and compound exocytosis[90], but rather occurs upon plasma membrane rupture during eosinophil cytolysis[90] (see *"Degranulation"*).

CLC/Gal-10 is observed in eosinophil-infiltrated tissue of patients with asthma, sinusitis, fungal allergic reactions, helminthic infections, myeloid leukemia[84,91], and during prolonged eosinophilic inflammatory reactions[84] (see *"Biomarkers"*). Furthermore, hypoxic conditions found in atopic patients promote CLC formation[92]. CLC/Gal-10 is found to trigger NLRP3 inflammasome, neutrophilic inflammation, type 2 sensitization, IgE synthesis, and granulogenesis during IL-5-driven eosinophil differentiation, as well as to interact with *EDN* and *ECP* for sequestration and vesicular transport[91,93].

Chemokines

Chemokines comprise a large family of *cytokines* involved in the regulation of cell movement (see *"Trafficking"*). They mainly act through the binding to G_i-protein-coupled seven-transmembrane *receptors* which leads to the activation of distinct intracellular signaling pathways[94] (Table 1). Chemokines are well known for taking part in the regulation of immune responses and the recruitment of immune cells[95] (see *"Immunoregulation"*).

A variety of chemokines are recognized to act on eosinophils and modulate their migration and *functions* (see *"Trafficking"*; Table 1). Especially the chemokine receptor C-C chemokine receptor (CCR)3 and thus its divers ligands are of high importance for eosinophil responses[94].

- Eotaxin 1 (CCL11) plays a role in the mobilization of mature eosinophils and eosinophil *progenitors* from bone marrow, eosinophil survival, eosinophil recruitment to tissue, eosinophil *degranulation*, *respiratory burst*, LTC_4 production, and lipid body formation[12,94,96-102].
- Eotaxin 2 (CCL24) is involved in eosinophil adhesion, and eosinophil recruitment to tissue[12,94,101,103].
- Eotaxin 3 (CCL26) contributes to eosinophil recruitment to tissue[94,104].

- MIP-1α (CCL3) induces eosinophil recruitment to tissue, eosinophil *degranulation*, and *respiratory burst*[94].
- RANTES (CCL5) plays a role in eosinophil recruitment to tissue, eosinophil *activation*, eosinophil *degranulation*, *respiratory burst*, LTC_4 production, and lipid body formation[94,98,99].
- MCP-2 (CCL8) triggers eosinophil recruitment, and eosinophil *activation*[94,105-108].
- MCP-3 (CCL7) contributes to eosinophil recruitment, and eosinophil *activation*[94,106,107,109].
- MCP-4 (CCL13) is involved in eosinophil recruitment, and eosinophil *activation*[94,106,110].

Moreover, human eosinophils express several chemokines themselves: CCL3/MIP-1α, CCL5/RANTES, CCL11/Eotaxin, CCL13/MCP-4, CCL17, CCL22, CCL23, C-X-C motif chemokine ligand (CXCL)1, CXCL5, CXCL8/IL-8, CXCL9/MIG, CXCL10, CXCL11, whereof only CCL5 and CCL11 are stored as preformed mediators in the secondary *granules*[95] (Table 3B). Human eosinophils are able to regulate T cell responses (see *"Immunoregulation"*) through the differential generation of Th1- or Th2-type chemokines in response to the environmental cytokines[111] (Figure 2). Furthermore, the previously listed eosinophil-derived chemokines are also involved in the recruitment and maintenance of tissue eosinophils (see *"Trafficking"*), as well as the promotion of eosinophil *functions*[95].

Cytokines

Cytokines are acting at extremely low concentrations through their specific *receptors* on target cells[112,113]. They can modulate functions of individual cells locally in autocrine, juxtacrine, and/or paracrine manners[113]. Furthermore, cytokines are involved in the regulation of immune responses (see *"Immunoregulation"*) through their action on various cells[95,112] (Figure 2).

Within the variety of cytokines that are known to activate (see *"Activation"*) and regulate eosinophil *functions*, IL-3, IL-5, and GM-CSF are regarded as the most important ones[102] (Table 1). They are involved in eosinophil proliferation and differentiation (see *"Origin"*), survival (see *"Apoptosis"*), adhesion, chemotaxis, migration (see *"Trafficking"*), and *degranulation*[114].

Furthermore, human eosinophils synthesize several cytokines themselves including GM-CSF, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-11, IL-12, IL-13, IL-16, IL-25/IL-17E, IFN-γ and TNF-α (Table 3C). A variety of these cytokines including IL-5, IL-6, IL-13, and TNF-α are found in the matrix or in the case of GM-CSF, IL-2, and IL-4 in the crystalline core of the secondary *granules* as preformed mediators[95]. On the other hand, *de novo* synthesis of cytokines is initiated by C5a, N-formyl-met-leu-phe (fMLP), inflammatory cytokines, and extracellular matrix (ECM) proteins[115].

Degranulation

Degranulation is a fundamental process in the *function* of eosinophils, during which *granule* content is released into the extracellular space upon stimulation[116]. To date, four major types of degranulation have been described in eosinophils[117]:

- Piecemeal degranulation: During PMD, the content of eosinophil secondary *granules* is selectively and progressively released via large and morphologically distinct membrane-bound tubular compartments designated as eosinophil sombrero vesicles (EoSVs)[118].

- Classical exocytosis: During classical exocytosis, the content of eosinophil secondary *granules* is released following the fusion of the granules with the plasma membrane[119].
- Compound exocytosis: In contrast to classical exocytosis, an additional step of intracellular granule-granule fusion occurs prior to fusion with the plasma membrane in compound exocytosis[119].
- Cytolysis: Cytolysis is a non-apoptotic form of cell death that is characterized by the release of intact *granules* from eosinophils following plasma membrane rupture.

The fusion of *granules* to either plasma membrane, already exocytosed granules, or intracellular granules was shown to involve distinct sensitivities to intracellular Ca^{2+} concentration and guanosine triphosphate (GTP) analogs revealing different regulatory mechanisms[80,120].

Eosinophil cationic protein

ECP, also known as RNase-3, is part of the ribonuclease A (RNase A) superfamily[121] (Table 3A). Its gene originated from a gene duplication with *EDN* as the second product, which led to the retrieval of cytotoxic activity but also partial loss of its ribonuclease activity[122]. Furthermore, ECP is found in association with the DNA scaffold of *EETs*[123]. In addition to *host defense*, tumor killing (see *"Cancer"*), and RNA degradation, ECP is involved in the nitration during *granule* maturation to enhance interaction between positively charged granule proteins after secretion[122]. ECP levels are used as a *biomarker* in various inflammatory diseases[122] (see *"Biomarkers"*).

ECP is mainly stored in an inactive form in secondary *granules* of eosinophils. Protein modifications involving deglycosylation during secretion lead to the generation of different ECP variants, each with a distinct molecular mass and cytotoxic activity, in an agonist-dependent manner[124]. The main cytotoxicity was exhibited by the ECP variants of 16.1 and 16.3 kDa[124]. Furthermore, ECP demonstrates amyloid aggregation capacity due to an N-terminus hydrophobic patch[125].

Eosinophil-derived neurotoxin

EDN, also known as RNase-2, is part of the RNase A superfamily, which also includes *ECP*[121] (Table 3A). Besides its RNA degrading action, EDN can mediate migration, maturation, and activation of DCs, enhances antigen-specific immune responses (see *"Immunoregulation"*), and is released in response to activation of eosinophils by specific bacterial species (see *"Host defense"*)[126,127]. Increased EDN levels are used as *biomarkers* for different inflammatory diseases[49,59,63]. It is worth noting that human EDN was also found extra-granularly in the cytoplasm as well as in association with the plasma membrane in addition to their location in the secretory *granules*[128].

Eosinophil extracellular traps

Upon stimulation, eosinophils can form extracellular structures known as EETs, which consist of granule proteins embedded in a mitochondrial DNA (mtDNA) scaffold[129]. EETs contribute to *host defense* against microorganisms such as bacteria, helminths, as well as fungi[123,130-133],

and are reported to take part in infectious skin diseases[45,130], allergic inflammatory diseases[130,134], and *autoimmune diseases*[130,131].

The exact mechanism of EET formation is still not well defined. The release of mtDNA from eosinophils has been observed to occur rapidly in a catapult-like manner independent of cell death[129]. More recently, it was suggested that the formation of EETs happens in the extracellular space, as the process of *degranulation* seems to take place ahead of mtDNA release[135]. The process of EET formation was demonstrated to rely on the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase through type I phosphoinositide 3-kinase (PI3K) activity (see *"Respiratory burst"*) and to be *autophagy*-independent[42]. The airway epithelial cell product surfactant protein-D (SP-D) was shown to prevent EET formation upon direct binding to the eosinophil cell membrane. However, this inhibition was lost in the oxidative environment during asthma exacerbations[136].

EET formation has also been observed in association with cell death where lytic cells release nuclear DNA[137,138]. A recent study reported extensive reactive oxygen species (ROS)-independent EET formation from lytic eosinophils in presence of a high concentration of endogenous lysophosphatidylserine (LysoPS)[139]. However, the requirement of cell death for the formation of extracellular traps is still a matter of scientific dispute[140].

Eosinophil peroxidase

EPX catalyzes a two-electron redox reaction using bromide, nitrite, or thiocyanate as substrate together with H_2O_2 and H^+ producing water and three possible products including hypobromous acid, nitrogen dioxide radical, and hypothiocyanous acid[141] (Table 3A). With its peroxidase activity, EPX participates in the *host defense* against various pathogens including parasites, bacteria, and viruses[142]. Additionally, EPX is a component of *EETs*[135]. Moreover, EPX exerts cytotoxic activity against tumors as well as host tissue[143,144]. EPX is found in the secondary *granules*[135].

Function

Historically, eosinophils are described as cytotoxic effector cells involved in *host defense* against various pathogens, as well as in pathophysiological processes including *allergies* and *autoimmune immunopathologies*[145] (Figure 3). Eosinophils defend against invading pathogens intracellularly by *phagocytosis* or through extracellular killing mechanisms such as *degranulation*, *EETs*, and *respiratory burst*[146]. Furthermore, eosinophils play a double-sided role in tumor control[69] (see *"Cancer"*). In addition, eosinophils are known to modulate immune responses through their interaction with a variety of immune cells such as T cells, MCs, DCs, and B cells[145,146] (see "*Immunoregulation"*). Moreover, eosinophils take part in *tissue homeostasis*, as well as *wound healing & tissue remodeling*[145].

Granules

Cytoplasmic granules are the hallmark of granulocytes, a subset of white blood cells (WBCs) formed by eosinophils, neutrophils, and basophils[147]. Granules form a storage site for many pre-synthesized pro-inflammatory factors including the granule proteins *MBP*, *ECP*, *EPX*, and *EDN*, as well as *cytokines*, *chemokines*, and *growth factors*[142,148]. Additionally, DNA and RNA are found in secondary granules[149].

Two distinct types of granules are observed in the cytoplasm of eosinophils[148] (see *"Morphology"*). Secondary granules, also termed specific or crystalloid granules, are formed during the myelocyte stage of eosinophil differentiation[150] (see *"Progenitors"*) and consist of a trilaminar membrane, a matrix, and a crystalloid core[148]. Larger core-free granules, originally named primary granules, are present in low numbers in the cytoplasm of eosinophils[148] and form during the progranulocyte stage[150]. In later years, these core-free granules were rather considered early secondary granules instead of a separate granule type, due to the occurrence of the unprocessed precursor of MBP (proMBP) whose mature form is mainly responsible for the formation of the crystalline core of the secondary granules[151]. *MBP* has to be stored in a non-toxic conformation in the crystalline core due to its extreme basicity and its ability to target different membranes[152,153]. On the other hand, proMBP was shown to carry a large number of acidic residues resulting in an almost neutral charge[152]. Therefore, proMBP is suggested to function as a cell protectant throughout the transport from the Golgi to the granules where it is cleaved to its mature form and safely stored[151]. Accordingly, proMBP is observed in eosinophilic promyelocytes, myelocytes, and metamyelocytes[151]. Moreover, condensed granules display proMBP in the periphery of the central area, which contrasts with the localization of MBP in the central area and suggest secondary granules as the site where proMBP is processed to MBP[151]. Furthermore, it was shown that IL-5 (see *"Cytokines"*) is sufficient to induce the production of proMBP, as well as the formation of mature MBP[151].

Growth factors

Eosinophils are able to synthesize and release several growth factors such as heparin-binding epidermal growth factor-like binding protein (HB-EGF-LBP), NGF, platelet-derived growth factor B chain (PDGF-B), stem cell factor (SCF), transforming growth factor (TGF)-α, TGF-β and vascular endothelial growth factor (VEGF)[95] (Table 3D). NGF, TGF-α, SCF, and VEGF are stored as preformed mediators in the matrix of secondary *granules*[95,154]. TGF-α is additionally found in small secretory vesicles[86,95]. Growth factors induce cell proliferation, as well as migration (see *"Trafficking"*), differentiation, and morphogenesis through their respective transmembrane *receptors* on target cells[155].

Host defense

Eosinophil granule proteins (Table 3A) exert a variety of anti-microbial mechanisms targeting different pathogens including helminths, bacteria, viruses, and fungi[142] (Figure 3). For instance, eosinophils produce α-defensin – a potent anti-microbial peptide reported to affect bacteria, parasites, and fungi[156].

- Bacterial infection: Eosinophils use several intracellular and extracellular mechanisms including *phagocytosis*, *degranulation* of diverse anti-bacterial mediators, and *EET* formation to kill bacteria[146,157].
- Fungal infection: Eosinophils can fight against fungal infection through the release of cytotoxic granule proteins, *cytokines*, and *chemokines*[142,146].
- Viral infection: Eosinophils act through the release of *cytokines* and granule proteins including ribonucleases[142,146], production of reactive nitrogen species[158] (see *"Respiratory burst"*), as well as promotion of T cell responses (see *"Immunoregulation"*) in the host defense against viruses[146]. Moreover, eosinophils are suggested to control neutrophil-mediated inflammation in COVID-19 infection[159]. Eosinopenia was observed in

hospitalized COVID-19 patients and therefore proposed as a prognostic indicator for the progression of more severe infection[160,161].

- Parasitic infections: Eosinophils can play both anti-pathogen and pathogen-supporting roles. Eosinophils provide host defense against parasites directly and through enhancement of adaptive immune responses by antigen presentation[142,146,156] (see *"Immunoregulation"*). However, eosinophils may also promote *Trichinella spiralis* infection by supporting larval growth and survival[162] and may reduce IL-4 response in Peyer's patches following re-infection with *Heligmosomoides polygyrus*[162].

Despite having a beneficial effect on the host defense, eosinophil cytotoxic effector *functions* may cause surrounding host tissue damage, which is thought to be limited when cytotoxic granule proteins are localized within mtDNA scaffold in the process of *EET* formation[135,146].

Hypereosinophilic syndromes

HES describes a spectrum of complex diseases characterized by persistent hypereosinophilia (HE) in peripheral blood (≥ 1'500 cells/mm^3) and/or tissue (see *"Biomarkers"*, *"Normal levels"*) associated with organ damage. For the diagnosis of HES, frequent secondary causes of eosinophilia are excluded[163,164] (Table 2). HE can result from a variety of factors including intoxications, infections particularly of parasitic origin, but also by bacterial and viral sources, medications such as anti-convulsants, antibiotics, and herbal supplements, as well as underlying conditions like *atopic diseases*, chronic inflammatory diseases, blood cell disorders and other neoplastic conditions[165,166]. Patients with HE can be asymptomatic for years but are prone to develop symptoms over time, eventually leading to HES[167]. Moreover, abnormal T cells that release IL-5 were identified in some patients with idiopathic HES, likely causing eosinophilia[168].

Recently, Valent *et al.* classified different types of HES according to their underlying etiologies[166]:

- Idiopathic HES describe HES of unknown etiology.
- Primary, or neoplastic, HES are characterized by a clonal myeloid or stem cell disorder.
- Secondary, also termed reactive, HES are defined by the expansion of activated eosinophils due to a related non-neoplastic or paraneoplastic condition.
- Several other variants of HES are known, including the lymphoid variant of HES which, in addition to the characteristics of secondary HES, displays abnormal T cell phenotype.

Therapies for HES aim to eradicate the underlying pathology or, at the very least, alleviate symptoms and prevent organ damage by controlling HE[166]. Possible strategies include corticosteroids, IFN-α, tyrosine kinase inhibitors, monoclonal anti-IL-5 or anti-IL-5 receptor antibodies, cytotoxic agents, and allogeneic stem cell transplantation[165,166] (see *"Treatment"*).

Immunoregulation

In addition to their cytotoxic effector *functions*, eosinophils are known to play a role in immunoregulation (Figure 2, 3). Through the release of preformed mediators stored in their *granules* (see *"Degranulation"*), human eosinophils are able to recruit C-X-C motif chemokine receptor (CXCR)2-expressing cells, such as neutrophils, to sites of inflammation[169], induce neutrophil activation and stimulate histamine release from basophils[170]. Furthermore, mouse

eosinophils were shown to trigger NK cell activation[170,171]. Within the MC/eosinophil interplay, eosinophils increase mediator release from basal MCs and contribute to the co-stimulation of IgE-activated MCs[172]. Moreover, the granule proteins *EDN* and *MBP* mediate the maturation and function of DCs[170]. Eosinophils were shown to increase peripheral B cell numbers[173], but also to promote B cell survival, proliferation, and Ig secretion[174]. Furthermore, eosinophils are involved in the maintenance of long-term survival of bone marrow plasma cells as well as intestinal mucosal IgA levels[174,175]. Additionally, mouse eosinophils modulate immune responses by shaping T cell polarization towards either Th2 or Th1[174,176-178]. A subset of circulating eosinophils expressing CD16 was demonstrated to suppress T cell responses mediated by *CLC*/Gal-10 in humans[179] (see *"Subpopulations"*). Moreover, eosinophils act as non-professional antigen-presenting cells (APCs) in allergic and parasitic inflammation[180,181]. Interestingly, mouse eosinophils are able to disrupt hematopoietic stem cell (HSC) homeostasis under conditions of eosinophilia[182].

Janus kinases

Janus kinases (JAK) are a family of cytoplasmic tyrosine kinases that comprises JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2)[183]. JAKs are associated with the cytoplasmic portion of various cytokine *receptors*, phosphorylating their intracellular domains upon activation[184]. JAK enzymes play a pivotal role in signal transmission, inducing biological responses[184]. Additionally, JAK2 is important in eosinophil differentiation through its association with IL-5Rα[185], implying that the eosinophil *activation* via IL-5 is JAK2-mediated[183]. Furthermore, JAK2 was shown to be critical for cytokine-mediated eosinophil survival through the JAK2/STAT pathway in humans[23] (see *"Apoptosis"*).

JAK enzymes are proven to be involved in several inflammatory diseases. Signaling of IL-4, IL-5, IL-13, and TSLP has been shown to occur through JAK1 and/or JAK2 in Th2-driven asthma[183]. Tofacitinib, a pan-JAK inhibitor originally considered as JAK3 inhibitor[186], is used for the *treatment* of rheumatoid and psoriatic arthritis, as well as ulcerative colitis[183]. Meanwhile, the JAK1/2 inhibitor baricitinib is applied in active rheumatoid arthritis, alone or in combination with methotrexate[183] (see *"Treatment"*). Both tofacitinib and baricitinib efficiently prevent eosinophil differentiation, activation, function, and survival[183].

Kinetics

Eosinophil numbers in peripheral blood are routinely used in the diagnosis (see *"Biomarkers"*) and *treatment* of eosinophil-associated diseases (Table 2). In physiological conditions, the absolute eosinophil count (AEC) accounts for 0.05-0.5 x 10^9/L[167] (see *"Normal levels"*). However, it is important to be aware of the variability in eosinophil numbers caused by many factors including circadian rhythm, season, exercise, diet, recent infection, and the use of corticosteroids[187,188]. Eosinophils' life span in the circulation averages 25h[189], but is prolonged up to 14 days in tissues[21,22] (see *"Apoptosis"*). Additionally, a marginated eosinophil pool was observed in the liver allowing the recirculation of eosinophils from a hepatic pool[189].

Eosinophil-targeted *treatments* interfere with eosinophil kinetics. Oral corticosteroids lead to decreased numbers of peripheral blood eosinophils within 1-2h after the start of the treatment[187,190]. Mepolizumab, an anti-IL-5 monoclonal antibody, induces immediate blood eosinopenia in eosinophilic asthmatic patients following treatment, whereas a complete restoration of eosinophil kinetics to homeostatic levels is observed even after long-term treatment[191]. The absence of IL-5 leads to retention and accumulation of eosinophils in the

bone marrow within less than 2 days of treatment explaining the rapid decrease of eosinophil numbers in the blood[191]. Surprisingly, the kinetic of mature eosinophils in blood and tissue was unchanged in response to the treatment suggesting that IL-5 blockade only decreases the number of dividing *progenitors* in the bone marrow, not their rate[191]. One week of treatment with dexamethasone was shown to inhibit IL-5-induced eosinopoiesis in a model of human umbilical cord blood mononuclear cells[192].

Lipid mediators

Upon *activation*, eosinophils release *de novo* synthesized lipidic arachidonic acid (AA)-derived mediators[13]. Furthermore, eosinophil lipid bodies form structurally distinct, inducible, non-nuclear sites for enhanced synthesis of paracrine eicosanoid mediators of inflammation, hence preserving key eicosanoid-forming enzymes such as cyclooxygenase, 5- and 15-lipoxygenase, and LTC_4 synthase[193] (Table 3E). Eosinophils are able to synthesize lipoxin A_4 (LXA_4), eoxin C_4 (C_4), thromboxane B_2 (TXB_2), prostaglandin (PG)E_2 and PGD_2 through oxidative metabolism of AA[13]. On the other hand, if eosinophils are properly stimulated, they mainly synthesize cysteinyl leukotrienes (CysLTs)[13]. CysLTs are involved in several eosinophil *functions*, including chemotaxis, survival, secretion, as well as differentiation, and maturation[194]. Moreover, both CysLTs *receptors* and P2Y12 receptor (P2Y12R) (Table 3F) are found on membranes of free eosinophil *granules*, implying their involvement in free granule secretory functions[194].

Major basic protein

In contrast to other cationic granule proteins, MBP is stored in the crystalline core of the secretory *granules* of eosinophils[153] (Table 3A). The toxicity of MBP is restricted by intragranular crystallization, whereas the amyloid aggregation propensity of MBP regulates its toxicity upon release in the extracellular space[153]. MBP plays an important role in *host defense* due to its anti-bacterial and anti-parasitic properties, as well as its involvement in *EET* formation[129,195,196]. Moreover, MBP exerts cytotoxicity against host tissue contributing to immunopathologic processes[153,197]. Additionally, proMBP plays a role as a proteinase inhibitor by covalently binding to pregnancy-associated plasma protein A (PAPP-A) through disulfide bond formation[198].

Mediator release

PMD represents the main secretory pathway of eosinophils for *cytokines* and *chemokines*[199] (see *"Degranulation"*). A variety of cytokines and chemokines are stored as preformed mediators in the secondary *granules* allowing a rapid release upon *activation*[95]. The selective and dynamic release of cytokines and chemokines in response to diverse types of stimulation implies a sorting mechanism for granule-stored mediators into secretory vesicles[95,117]. Loading of the vesicles is suggested to either occur through the fusion of pre-existing cytoplasmic vesicles to eosinophil granules or through the formation of new vesicles by budding from granule membranes[200,201]. Moreover, IL-4 was observed in secretory vesicles together with IL-4Rα, implying receptor-mediated trafficking of eosinophil-derived cytokines[117]. Furthermore, the release of CCL5/RANTES is proposed to be mediated by vesicle-associated membrane protein (VAMP)-2 and its interaction with synaptosome-associated protein 23 (SNAP23) and syntaxin-4[202]. Additionally, eosinophils use exocytosis to release preformed cytokines (see

"Degranulation"), in contrast to newly formed cytokines which are secreted through the constitutive pathway – a process describing the vesicular transport from the endoplasmic reticulum (ER) and Golgi apparatus towards the plasma membrane[199].

Metabolism

Human eosinophils use glycolysis, glucose oxidation, and oxidative phosphorylation demonstrating their metabolic flexibility[203] (Figure 4). Glucose uptake in human eosinophils is regulated by the level of intracellular Ca^{2+}, the mitogen-activated protein kinase (MAPK) pathway, as well as various glucose transporters (GLUTs)[54]. However, human eosinophils are less responsive to enhanced glucose in contrast to neutrophils[203,204], as increased glucose uptake was only observed after IL-5, IL-3, GM-CSF, or TNF-α stimulation[54,205]. Nevertheless, the glucose uptake capacity of lung eosinophils has been shown to reduce NK cell anti-tumoral functions by decreasing glucose levels in the tumor microenvironment[206] (see *"Cancer"*). Glucose oxidation is used as an alternative energy pathway to glycolysis in humans, during which glucose-derived pyruvate enters mitochondria and subsequently participates in the tricarboxylic acid (TCA) cycle[203]. ROS production (see *"Respiratory burst"*) resulting from mitochondrial respiration is important for the activation of ERK1/2 and caspase-3 in humans and thus may result in eosinophil adhesion, cellular damage, and accelerated *apoptosis*[207]. Inhibition of ROS and subsequent reduction of TCA cycle intermediates results in increased glucose-derived lactate production highlighting the metabolic plasticity of human eosinophils[205]. Likewise, human eosinophils shift to the glycolytic pathway in hypoxic conditions, where mitochondrial oxidative phosphorylation is suppressed[203].

Morphology

Eosinophils were first discovered in 1846 by Wharton Jones[208]. However, Paul Ehrlich gave eosinophils their name in 1879 because of their ability to be stained by the acidic dye eosin[208]. Eosinophils are round-shaped WBCs with a diameter of 10 to 20 µm and generally have a bilobed nucleus[209]. Eosinophils together with neutrophils and basophils belong to the granulocytes – a group of WBCs characterized by the high abundance of *granules* in their cytoplasm, in which they can store preformed mediators[147]. Eosinophils are largely conserved in their morphology and their response to helminth antigens (see *"Host defense"*) throughout all vertebrate species[208,210,211] (see *"Zebrafish"*).

Necroptosis

Necroptosis is an inflammatory type of regulated cell death defined by a necrotic phenotype such as cell swelling and plasma membrane rupture resulting in the release of danger-associated molecular patterns (DAMPs), *cytokines*, and *chemokines*, that will ultimately trigger an inflammatory response[212]. Besides *apoptosis*, human and mouse eosinophils were lately reported to selectively undergo necroptosis in the lungs of asthmatic patients and a mouse model of asthma[213]. Eosinophil necroptosis is mediated through necroptotic components including the receptor-interacting protein kinase (RIPK)1, RIPK3, and the mixed lineage kinase-like protein (MLKL) and was shown to play a role in airway inflammation, remodeling, and hyperresponsiveness in asthma[213].

Another study demonstrated the involvement of RIPK3 and MLKL, but not RIPK1, in adhesion-induced cytolysis of human eosinophils – a process shown to be counterregulated by

autophagy[214]. The activation of p38 MAPK and PI3K downstream of the RIPK3-MLKL pathway results in ROS production, cytoplasmic vacuolization, and ultimately cytolysis[214].

Normal levels

Under homeostatic conditions, eosinophils make up for 1-5% of total circulating leukocytes[188,215-217]. The AEC defines the number of circulating eosinophils in human peripheral blood and ranges between 0.05-0.5 x 10^9/L under normal conditions[167] (see *"Kinetics"*). It is worth noting that the blood eosinophil count varies by around 20% during the day[187,188]. Eosinophilia is defined by an AEC exceeding 500 cells/µL, while the AEC threshold characterizing hypereosinophilia is 1500 cells/µL (see *"Biomarkers"*)[167,188,218]. The underlying cause of eosinophilia is used to classify eosinophilic disorders into intrinsic/primary disorders, which are characterized by mutations in pluripotent hematopoietic or multipotent myeloid stem cells, and extrinsic/secondary disorders, where released cytokines induce eosinophilia[215].

Tissue eosinophils are found under physiologic conditions in the thymus, mammary glands, uterus, and the non-esophageal part of the gastrointestinal tract[167,188,216] (see *"Tissue homeostasis"*). No pathologic threshold is defined for tissue eosinophils, but the increase in eosinophil number at the above-mentioned sites, as well as the presence of eosinophils at other anatomic sites, is observed under pathologic conditions[188] (see *"Biomarkers"*).

Origin

In the course of hematopoiesis, mouse HSCs give rise to common myeloid *progenitors* (CMPs) that further differentiate into granulocyte-macrophage progenitors (GMPs) and finally eosinophil-lineage committed progenitors (EoPs)[219] (Figure 5A). In contrast, human EoPs arise from CMPs independently of GMPs[220]. Additionally, an IL-33-mediated differentiation from GMP-like to eosinophil precursors (EoPres) through IL-33 receptor ST2 has been described[221]. EoPs further differentiate into myeloblasts, promyelocytes, and myelocytes before ultimately reaching the final mature eosinophil stage under the influence of IL-3, IL-5, and GM-CSF[20] (see *"Cytokines"*) (Figure 5B).

The dynamic and hierarchical action of different sets of transcription factors (TFs), as well as soluble mediators is crucial for the eosinophil lineage commitment[222,223] (Figure 5).

- Role of C/EBP members: Stimulation of eosinophil differentiation; transient expression leads to eosinophil lineage commitment and thus the formation of immature eosinophils; sustained expression stimulates maturation of eosinophils
- Role of PU.1: Switch from lymphoid to myeloid lineage; high levels increase myeloid differentiation; synergistic activity with GATA-1 to regulate eosinophil lineage specification & granule protein transcription
- Role of GATA-1: Essential for eosinophil lineage commitment
- Role of IL-3, IL-5, GM-CSF: Synergize towards differentiation of eosinophils
- Role of IL-5: Is the most specific of the cytokines to eosinophil lineage; stimulates the release of eosinophils from the bone marrow into the peripheral circulation

Phagocytosis

Among other effector *functions*, eosinophils exhibit phagocytic capabilities. Although they are not as efficient as macrophages or neutrophils, human eosinophils have been reported to be able to ingest pathogens like *Trypanosoma dionisii*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*[224,225]. The process of eosinophil phagocytosis requires glycolysis and protein synthesis but does not rely on oxidative *metabolism*[224]. Eosinophil phagocytosis was shown to be CD35-dependent (see *"Surface markers"*) in humans, whereas neutrophil phagocytosis was mediated by CD16 and CD32[226]. More recently, eosinophil phagocytosis was shown to be slightly impaired following treatment with inhibitors of JAK1/2 (baricitinib) and JAK3 (tofacitinib) in eosinophilic asthma patients [183] (see *"Janus kinases"*).

Progenitors

Eosinophils develop from HSCs through intermediate progenitors (see *"Origin"*) that are characterized by differential expression of lineage-associated *surface markers*[219,227] (Figure 5):

- mHSC: c-Kit$^+$, Lin$^-$, Sca-1$^+$

  hHSC: c-Kit$^+$, Lin$^-$
- mCMP : Sca-1$^-$, IL-7Rα$^-$, IL-5Rα$^-$, FcγRII/III^{lo}, CD34$^+$

  hCMP: IL-5Rα$^-$, IL-3Rα$^+$, CD45RA$^-$, CD34$^+$, CD38$^+$
- mGMP: IL-5Rα$^-$, FcγRII/III$^+$, CD34$^+$
- mGMP-like: Siglec-F$^+$, IL-5Rα$^-$, CD34$^+$, ST2$^+$
- mEoP: c-Kit^{lo}, IL-5Rα$^+$, CD34$^+$

  hEoP: c-Kit$^-$, IL-5Rα$^+$, IL-3Rα$^+$, CD45RA$^-$, CD34$^+$, CD38$^+$
- Myeloblast: IL-5Rα$^+$, CD34$^+$
- Promyelocytes: CD11b$^-$, CD62L$^-$, CD193^{low}, siglec-8^{low}
- Myelocytes: CD11b^{high}, CD62L$^-$, CD193^{low}, siglec-8^{low}
- Mature eosinophils: CD11b^{high}, CD62L^{high}, CD193^{high}, siglec-8^{high}

In the course of eosinophil-related airway diseases, EoPs are observed in cord blood, peripheral blood, lung tissue, and sputum in addition to their main location in the bone marrow[228,229] (Table 2). Following allergen exposure (see *"Allergy"*), mature and immature eosinophils, as well as eosinophil-committed progenitors are released from the bone marrow and recruited to inflammatory tissues[228]. Moreover, higher levels of EoP in peripheral blood were observed in patients with active EoE, suggesting its possible use as a *biomarker* in this disease[230].

Q fever

Eosinophils are involved in *host defense* against pathogens and were shown to play a role in Q fever – a worldwide distributed zoonosis caused by the obligate intracellular gram-negative bacterium *Coxiella burnetii* (*C. burnetii*)[231]. Patients with acute Q-fever may experience flu-like symptoms, fever, pneumonia, and hepatitis, while chronic Q-fever may result in the development of endocarditis, stillbirth, and some less frequent complications[231].

Pleural fluid eosinophilia was observed in different *C. burnetii*-infected patients and is generally indicative of a favorable outcome[232] (see *"Biomarkers"*, *"Normal levels"*). The presence of fibrillar eosinophilic structures was observed in granulomatous hepatic lesions of patients with Q-fever[233]. More recently, a case of acute Q fever with pancreatitis, hypereosinophilia, and eosinophilic alveolar infiltrates has been reported, suggesting a role for eosinophils in the pathogenesis of the disease[234]. In mice, vaccination against *C. burnetii* led to eosinophil accumulation mediated by $CD4^+$ T cells in the spleen[235]. Furthermore, eosinophils were attributed a protective effect in early vaccine protection through their involvement in the IFN-γ response, which contributes to *C. burnetii* clearance through antibody isotype switching and activation of Th1 cells[235] (see *"Immunoregulation"*).

Receptors

The large variety of surface molecules and receptors present on eosinophils reflects the wide range of *functions* they perform (Table 3F).

- Pattern recognition receptors (PRRs): Toll-like receptor (TLR)1-7, TLR9, TLR10 nucleotide-binding oligomerization domain-like (NOD) 1, NOD2, dectin-1, and receptors for advanced glycation endproducts (RAGE)[236-238]. They are activated by pathogen-associated molecular patterns (PAMPs) or DAMPs[238] and are involved in survival (see *"Apoptosis"*), *respiratory burst*, activation of the adhesion system (see *"Trafficking"*), and the release of *cytokines*, *chemokines*, and granule proteins[238,239].
- Adhesion receptors: Integrins, L- and P-selectins. They are mediating the adhesion of eosinophils to surrounding cells or ECM[102] (see *"Trafficking"*).
- Cytokine receptors: IL-1R, IL-2Rα/β/γ chains, IL-3Rα chain, IL-5Rα chain, GM-CSFR α chain, IL-4Rα/γ chains, IL-9R, IL-10R, IL-12R, IL-13R, CD4, IL-17R, IL-23R, IL-17BR, IL-27R, ST2, IFN-γR, TNF-αR, TSLPR. Additionally, IL-5R, IL-3R, and GM-CSFR share a common β chain in addition to their respective α chains. Irreversible binding of the *cytokines* to their respective receptors leads to the transduction of their signals and resultant intracellular changes such as gene expression, cell cycle changes, and *mediator release* (see *"Activation"*). Cytokine receptors are formed as oligomeric complexes of two to four receptor chains[102,115,240].
- Chemokine receptors: CCR1, CCR3, CCR4, CCR5, CCR6, CCR8, CCR9, CXCR2, CXCR3, CXCR4. Activation of chemokine receptors leads to cell migration and adhesion via signaling through $G\alpha_i$ G-proteins and β-arrestins[102,241] (see *"Trafficking"*).
- Growth factor receptors: PDGFR, EGFR, Trk-B. *Growth factor* receptors transmit their signal by receptor tyrosine kinase or receptor serine-threonine kinase[102,242-246].
- Lipid mediator receptors: $CysLTR_1$, $CysLTR_2$, BLT_1, BLT_2, PAFR, DP_1, DP_2. These receptors induce eosinophil functional responses (see *"Function"*, *"Lipid mediators"*) and are mostly part of the G-protein coupled receptors (GPCRs) except for DP_1 and DP_2. DP_1 acts through Gαs protein and subsequent increase in cyclic adenosine monophosphate (cAMP), while DP_2 signals via $G\alpha_i$ and leads to an increase in intracellular calcium, decrease in cAMP, and activation of PI3K[13,102,247,248].
- GPCRs: Their ligands comprise PAF, prostanoids, leukotriene B_4 (LTB_4), CysLTs, LXA_4, chemokines, complement components, neuropeptides, *N*-formylated methionyl peptides, purines, and pyrimidines, as well as ethylamines and catecholamines. Their function is mediated through G-proteins[249].

- Fc receptors: FcαRI, FcDR, FcγRII, FcμR. They are engaged in interaction with the adaptive immune system (see *"Immunoregulation"*) and, in the case of FcαRI and FcγRII, in eosinophil *activation*[102].
- Major Histocompatibility Complex-II (MHC-II): It mediates antigen presentation in combination with the co-stimulatory molecules CD80 and CD86[102] (see *"Immunoregulation"*).
- Siglecs: human eosinophils express siglec-3 (CD33), -7, -8, and -10 (see *"Biomarkers"*, *"Surface markers"*); mouse eosinophils express siglec-F, CD22 (i.e. siglec-2), siglec-E, and siglec-G. Siglec-8 and siglec-F are involved in eosinophil survival (see *"Apoptosis"*), while siglec-7 inhibits *degranulation* and *cytokine* release. The functions of siglec-3, siglec-10, CD22, siglec-E, and siglec-G in eosinophils are not yet known[102,250-253].
- Soluble NSF attachment protein receptor (SNARE): VAMP-2, VAMP-7, VAMP-8, SNAP23, syntaxin-4, syntaxin-17. SNARE proteins mediate the fusion of *granules* or vesicles to each other or membranes[119,254,255] (see *"Mediator release"*).

Respiratory burst

Eosinophils are able to produce and release ROS through NADPH oxidase – a complex composed of distinct subunits localized in the cytosol ($p40^{phox}$, $p47^{phox}$, $p67^{phox}$, and Rac GTPase) and the plasma membrane ($p22^{phox}$ and $gp91^{phox}$)[256]. The rapid release of ROS, also referred to as respiratory burst or oxidative burst, was reported to occur in response to infectious agents[146] (see *"Host defense"*). Moreover, eosinophils produce high levels of ROS in allergic diseases and inflammatory disorders such as asthma, allergic rhinitis, and eosinophilic pneumonia which may ultimately lead to tissue damage[257-259]. Although ROS has been reported to be harmful to lipids, proteins, and DNA, a low level of ROS is crucial for the cell to maintain a normal *metabolism*, signal transduction, cell growth, and undergo *apoptosis*[260]. Human eosinophils have been shown to generate larger amounts of ROS extracellularly as compared to neutrophils, which preferentially produce ROS intracellularly[258]. The respiratory burst in chemotaxin-stimulated eosinophils has been shown to rely on intracellular calcium flux and to be inhibited by β-adrenoceptor agonists in humans[261,262]. Furthermore, cross-linking of siglec-8 was reported to induce intracellular ROS production, as well as caspase cleavage and a decrease in mitochondrial membrane potential, resulting in *apoptosis* in resting human eosinophils[260]. Interestingly, siglec-8 cross-linking on IL-5-primed eosinophils has been shown to induce ROS production along with increased phosphorylation of MEK1 and ERK1/2, leading to caspase-independent necrotic death, while the cross-linking of siglec-8 on IL-33-primed eosinophils resulted in enhanced cell death without ROS production[260]. Moreover, cross-linking of siglec-F on mouse eosinophils induced caspase-dependent cell death without ROS production[260]. Ultimately, it was shown that ROS production is essential in the process of *EET* formation[129].

Subpopulations

The discovery of hypodense circulating eosinophils in patients with different diseases in the early 1980s led to the first speculations regarding eosinophil heterogeneity[263] (Figure 6). In later years, it was shown that eosinophils that differentially express *cytokines* exhibit distinct functional roles in skin diseases[264].

In mice, two phenotypically and functionally distinct eosinophil subpopulations – resident eosinophils (rEos) and inflammatory eosinophils (iEos) – were identified in the lung tissue[265]. These results were also relevant to humans as rEos in parenchymal lung tissue of healthy patients and iEos in sputa of eosinophilic asthmatic patients were shown to exhibit different surface phenotypes[265]. Eosinophil subsets with distinct cytokines expression and differential surface expression of Gr-1 have been described in mouse lung tissues[266]. Furthermore, a CD11c^{hi} subset of eosinophils (see *"Surface markers"*) was identified in the mouse intestine that differs phenotypically from blood eosinophils and eosinophils from lamina propria[267]. Interestingly, it has been demonstrated that the transcription factor aryl hydrocarbon receptor (AHR) partially controls the transcriptomic changes in the process of tissue adaption of mouse intestinal eosinophils[268]. Interestingly, AHR was reported to drive the expression of immunomodulatory features such as the C-type lectin domain family 4, member a4 (Clec4a4) – a C-type lectin inhibitory receptor – and the inhibitory ligand programmed death-ligand 1 (PD-L1) in a distinct subset of blood-derived mouse eosinophils exclusively found in the lamina propria of the small intestine[269]. Moreover, different mouse eosinophil subsets were categorized as *progenitors*, steady state eosinophils, and type 1 and 2 activated phenotypes implying that different tissue microenvironments shape eosinophil phenotype and *function*[270]. In accordance, a recent study identified five eosinophil subpopulations including eosinophil precursors, immature, circulating, basal, and active eosinophils across mouse tissues based on single-cell transcriptomic profiling[271].

Surface markers

Eosinophils express several surface proteins that enable their identification. To distinguish eosinophils from neutrophils, the cells are generally examined for their lack of CD16 expression and their presence of CD49d expression[272]. Furthermore, eosinophils are known to express CD61 and CD193[227].

The differential expression levels of surface proteins are used to identify the maturation stages of human eosinophils[227] (see *"Origin"*, *"Progenitors"*):

- CD11b, CD62L, CCR3 (CD193), siglec-8: Upregulation during maturation
- CD64: Low expression in promyelocyte stage
- LAIR1 (CD305): Expressed during all stages of maturation, but highest levels are found during promyelocyte and myelocyte stage
- CD49d, CD66b: Maintained during maturation, but peak levels occur during the myelocyte stage
- CD125: Expressed during all stages of maturation, but the highest levels are found in mature eosinophils
- CD35: Expressed only in mature eosinophils

Upon *activation*, the expression level of human eosinophil surface markers is changed. CD62L, CD23, CD31, and PSGL-1 levels are decreased, while levels of CD35, CD11b, CD66, CD69, CD81, and CD44 are increased in activated eosinophils[17,273]. On the other hand, the expression of CD9, CD49d, siglec-8, and CD11a remains stable[17]. Moreover, endothelial cells are shown to increase expression levels of CD69 and CD35 mediating eosinophil migration from blood to tissue in humans[274] (see *"Trafficking"*). Furthermore, eosinophils from patients

suffering from food *allergy* with eosinophilia express increased levels of CD23, CD44, CD45, and CRTH2, which are markers of eosinophil *activation*[275]. An increased surface level of CD25 led to an increased risk of eosinophil *degranulation* and was observed in patients with eosinophilia and Wells' syndrome[116].

Tissue homeostasis

In humans and mice, eosinophils executing homeostatic *functions* are commonly found in the gastrointestinal (GI) tract, adipose tissue (AT), uterus, thymus, and lungs[276] (see *"Normal levels"*) (Figure 3). Additionally, in mice, eosinophils have been reported to home to mammary *glands*[167,188,216,277].

Under physiologic conditions, human and mouse eosinophils are present in the GI tract except for the esophagus. In mice, GI tract eosinophils have been shown to regulate IgA and cellular T cell responses promoting homeostatic immunity[276,278] (see *"Immunoregulation"*). Similarly, such homeostatic eosinophils negatively regulate Th2 cell response and maintain immune homeostasis in human and mouse lungs[276]. Interestingly, eosinophils seem to be able to proliferate locally in the human thymus and contribute to thymocyte selection and maturation[279]. In AT of mice, eosinophils are the main source of IL-4 (see *"Cytokines"*), which plays a crucial role in alternative macrophage activation, a marker of healthy AT[280]. These eosinophils are therefore involved in the maintenance of an anti-inflammatory milieu and regulate tissue homeostasis in visceral white AT in obesity-associated metabolic disease as well as aging in mice[174,281]. Furthermore, the production of IL-4 by mouse eosinophils drives adipocyte beiging and consequently promotes thermoregulation in white AT through the IL-4Rα-STAT6 signaling pathway in M2 macrophages[174]. Additionally, eosinophils in mouse AT have been shown to contribute to glucose homeostasis and energy consumption[282] (see *"Metabolism"*). Moreover, it has been demonstrated in mice that eosinophils directly affect perivascular AT (PVAT) function through the release of catecholamines independently of other immune cells[283]. Contrarily, enhanced numbers of eosinophils in subcutaneous AT (SAT) have also been associated with increased inflammation and tissue dysregulation in patients with metabolic syndrome (MetS)[284]. In addition, eosinophils have been reported to significantly improve cardiac dysfunction, cell death, and fibrosis following myocardial infarction[285].

Trafficking

Terminally differentiated eosinophils are released into the circulation where they get polarized in response to immunological stimuli and chemotactic factors (see *"Activation"*, *"Chemokines"*), inducing shape changes and migration to inflamed tissues[286] (Table 1).

The transmigration of eosinophils through the vascular endothelium requires rolling, tethering, firm adhesion, and transendothelial migration[287]. Rolling on the endothelium is mediated by L-selectins that are constitutively expressed on eosinophils (see *"Receptors"*) and endothelial-expressed L-selectin ligands[286]. E- and P-selectins found on the endothelium interact with their respective ligands on eosinophils leading to the tethering of eosinophils to endothelium[286]. Finally, the interaction between eosinophil adhesion molecules and endothelial adhesion receptors together with *chemokines* and *cytokines* allows a firm adhesion and transmigration of eosinophils into tissues[286].

Eosinophils constitutively express the chemokine *receptors* CCR3 and CCR1, as well as several other chemokine receptors such as CXCR3, CXCR4, CCR5, CCR6, and CCR8 upon

stimulation with IL-5[286] (Table 1). *Chemokines* can shift beta1 integrins towards beta2 integrins facilitating eosinophil migration[103] and promoting eosinophil *activation* and *function*[288].

Treatment

To date, several distinct treatments are commonly used for eosinophil-associated inflammatory diseases including glucocorticoids and agents against IL-5, IL-5Rα, IL-4, and IL-13 (Table 2).

Glucocorticoids are considered as one of the most effective therapies for eosinophilic disorders[32]. They have been proposed to reduce eosinophil numbers in blood and tissue by three mechanisms[289-291] (see *"Apoptosis", "Kinetics"*):

- Inhibition of the synthesis of pro-survival signals of eosinophils such as IL-3, IL-5, and GM-CSF
- Eosinophil clearance through direct induction of apoptosis
- Eosinophil clearance through professional phagocytic cells

However, glucocorticoids are known to cause pleiotropic effects which potentially lead to unwanted side effects[32].

The IL-5-targeting agent mepolizumab effectively reduces the number of circulating eosinophils but is less potent in eliminating tissue eosinophils and does not reduce the numbers of eosinophil *progenitors* in the bone marrow[32,292,293] (see *"Normal levels"*, *"Kinetics"*). Mepolizumab is a humanized IgG1 monoclonal anti-IL-5 antibody that blocks the binding of IL-5 to its respective receptor IL-5Rα by interfering with its ligation to the α-chain[48]. It is administered subcutaneously and is used in the treatment of asthma, EGPA, *HES*, chronic rhinosinusitis with or without nasal polyps, and EoE[32,48] (Table 2). Furthermore, reslizumab, a humanized IgG4 anti-IL-5 antibody, is approved for intravenous treatment of asthma, *HES*, EoE, and nasal polyposis[32,48] (Table 2).

Benralizumab is a humanized afucosylated IgG1 monoclonal anti-IL-5Rα antibody[32,48]. It binds to the Fab domain of the IL-5Rα therefore blocking the binding of IL-5 to its receptor[48]. Benralizumab inhibits eosinophil proliferation, differentiation, and maturation in the bone marrow[48,294]. Additionally, benralizumab induces ADCC in eosinophils via the binding of its afucosylated Fc domain to the RIIIa region of the Fcγ found on NK cells, macrophages, and neutrophils[48]. This dual function causes a stronger and faster depletion of eosinophils as compared to treatments with mepolizumab and reslizumab[48,295]. In addition to its strong effect on blood eosinophils, benralizumab was also shown to reduce tissue eosinophil numbers, although it was subjected to individual variability[48] (see *"Normal levels"*).

Dupilumab is a human IgG1 anti-IL-4Rα antibody that blocks the binding of both IL-4 and IL-13 to its receptor, resulting in the inhibition of IL-4- and IL-13-induced responses such as the release of pro-inflammatory *cytokines*, *chemokines*, and IgE[48]. Dupilumab is an established treatment for AD, asthma, and chronic rhinosinusitis with or without nasal polyps[48,296] (see *"Atopic diseases"*). On the other hand, it should be noted that transient eosinophilia is a known side effect[297] (see *"Normal levels"*).

A humanized IgG4 anti-IL-13 antibody, lebrikizumab, is currently tested in phase 3 clinical studies for the treatment of AD[48] (see *"Atopic diseases"*).

Unexplained eosinophilia

Unexplained eosinophilia describes untreated patients with persistent asymptomatic eosinophilia that don't demonstrate clinical manifestations of *HES*[298,299]. In patients with unexplained eosinophilia, the presence of eosinophil-mediated end-organ damage should be considered the highest priority to start immediate therapy if required[300]. The proposed *treatment* of choice is corticosteroids[300].

Possible underlying causes, such as clonal eosinophilia, drug hypersensitivity, and occult malignancy, should be examined in patients with unexplained eosinophilia[300]. Moreover, chronic unexplained eosinophilia is a well-known characteristic of PDGFRA-associated chronic eosinophilic leukemia[301]. It is important to note that demographics, routine laboratory testing, and measurements of eosinophil *activation* or *cytokines* profiles do not differ between unexplained eosinophilia, untreated symptomatic idiopathic *HES*, and lymphocytic variant *HES* impeding the correct diagnosis[298].

Furthermore, hypereosinophilia of undetermined significance (HE_{US}) is characterized by unknown etiology of the HE, negative family history, absence of reactive or neoplastic condition or disorder underlying the HE, and the absence of signs or symptoms indicative of *HES*[299]. Moreover, it requires the exclusion of any underlying disease or condition that might trigger HE, in addition to ruling out *HES*[299].

Vasculitis

EGPA, previously referred to as Churg-Strauss syndrome, is a rare Th2-mediated pathology that is associated with asthma and eosinophilia[302] (see *"Autoimmune diseases"*). It is defined by blood vessel inflammation (i.e. vasculitis) and eosinophil-rich granulomatous inflammation[302]. Tissue-infiltrating eosinophils are thought to play a role in the pathophysiology of the disease[303,304]. Two genetically distinct groups of EGPA were suggested as a result of a genome-wide association study: anti-neutrophil cytoplasm antibody (ANCA)-positive and ANCA-negative EGPA[305]. The absence of ANCA autoantibodies is associated with increased mortality, lower risk of relapse, reduced vasculitis manifestation, and increased cardiomyopathies[306].

The anti-IL-5 monoclonal antibody mepolizumab has been shown to be clinically efficacious for the *treatment* of EGPA. It has been approved by the FDA and is used add-on to glucocorticoids, which used to be a long-established option to treat EGPA[307]. Depending on the disease severity, drug combinations can be used, comprising corticosteroids, disease-modifying anti-rheumatic drugs, and biological agents[308].

Wound healing & tissue remodeling

Eosinophils are known to play a role in wound healing and tissue remodeling (Figure 3). Damaged cells release DAMPs that activate eosinophils through their PRRs (see *"Receptors"*) mediating their migration to sites of tissue injury and necrosis (see *"Trafficking"*)[208]. In mouse skeletal muscle and liver tissue, eosinophils have been shown to promote tissue healing through IL-4 production[174]. In human skeletal muscle injury, eosinophils contribute to wound healing by promoting cell proliferation with the help of released *cytokines*, including IL-4, VEGF, fibroblast growth factor (FGF), TGF-β1, and osteopontin[208] (see *"Growth factors"*). Additionally, eosinophils mediate the formation of fibrin through fibrinolysis in coagulation pathways, trigger the production of remodeling factors by epithelial cells, and assist with ECM

deposition[309]. Moreover, the catalytic activity of *EPX* upon internalization into human osteoblasts is shown to promote collagen I biosynthesis, and matrix mineralization, as well as to regulate osteogenic gene expression in new bone formation subsequent to a bone fracture or trauma[310].

X-inactivation

X chromosome inactivation describes the process of transcriptional inactivation of one of the X chromosomes in females during embryogenesis. Some genes can escape from this process including *CD40L*, *CD99*, *LAMP-2*, *IRAK-1*, *TLR7*, *USP27X*, *DDX3X*, *CXORF21*, and *XIAP* that are shown to be involved in autoimmunity (see *"Autoimmune diseases"*)[311].

The clonal pattern of X-inactivation was observed in eosinophils of a female patient with idiopathic *HES* indicating a low-grade clonal disorder as the cause of the eosinophilia in contrast to reactive eosinophilia[312]. Moreover, the combination of skewed X-inactivation with T cell receptor gene rearrangement, fluorescence *in situ* hybridization analysis, and chromosomal karyotyping contributed to the accurate diagnosis of two unusual cases of pediatric hypereosinophilia[313].

X-rays

X-rays can be of help in the diagnosis of eosinophil-related diseases through the identification of characteristic infiltrations in the lung:

- Chronic eosinophilic pneumonia: Migratory peripheral infiltrates[314]
- Idiopathic chronic eosinophilic pneumonia: Bilateral infiltrates with mixed alveolar or interstitial opacities[315], peripheral pulmonary opacities[316]
- EGPA: Lung opacities with ill-defined pulmonary infiltrates[315]
- Tropical pulmonary eosinophilia: Bilateral disseminated opacities[317]
- Visceral larva migrans syndrome: Pulmonary infiltrates[318]
- Strongyloidiasis: Bilateral patchy infiltrates[315]
- Drug-induced eosinophilic pneumonia: Transient pulmonary infiltrates[315]

Y-box-binding factor

Y-box-binding factor 1 (YB-1) is a DNA- and RNA-binding protein that is involved in many DNA- and mRNA-dependent processes[319]. Human YB-1 enhances eosinophil survival through its interaction with GM-CSF mRNA, resulting in the stabilization of GM-CSF mRNA upon *activation*[320] (see *"Apoptosis"*, *"Cytokines"*). The interaction of YB-1 with GM-CSF mRNA is supposed to prevent RNAse recognition or cleavage[321].

Zebrafish

Eosinophils characterized as *gata2^{hi}* cells in adult hematopoietic tissues have been identified in zebrafish, demonstrating the high conservation of this immune effector cell lineage through evolution[210]. In contrast to human eosinophils, zebrafish eosinophils demonstrate an

eosinophilic cytoplasm with numerous peroxidase-negative *granules* and a small, non-segmented, peripherally located nucleus[322,323] (see *"Morphology"*). Eosinophils are found in the kidney marrow and the peritoneal cavity of zebrafish[210]. The conserved role of the immune system throughout evolution allows the use of zebrafish as a model organism in eosinophil research[158,324-326].

Conclusion and perspective

The growing interest in eosinophils over the last 30 years contributed to a deeper understanding in the function of eosinophils in health and disease. Besides their original description as cytotoxic effector cells engaged in host protection, eosinophils are nowadays well recognized for their involvement in immunoregulation and tissue homeostasis.

Owing to their involvement in the pathogenesis of allergic and non-allergic inflammatory diseases, eosinophils represent a promising therapeutic target. In accordance, eosinophil count and levels of eosinophil-related mediators are used as relevant biomarkers. Additionally, the prevalence of eosinophils in the tumor microenvironment was associated with the outcome of distinct cancer types and therefore represents a promising predictive factor.

Glucocorticoids and monoclonal antibodies targeting soluble mediators associated with eosinophilic inflammation such as IL-4, IL-13, IL-5, and its receptor IL-5R are common eosinophil-targeting therapies that aim to reduce eosinophil numbers and alleviate patients' symptoms.

Emerging evidence proposes a high plasticity potential for eosinophils allowing them to adapt their phenotype and function to the microenvironment. While the variation across the different studies emphasizes the heterogeneity of these cells, it simultaneously impedes the identification of recurrent subgroups.

A greater knowledge and understanding of the biology of eosinophils and their contribution in homeostatic and inflammatory processes in pathophysiological conditions remains a requirement for the development of novel diagnostic and therapeutic strategies for patients with eosinophil-related disorders.

Abbreviation

AA	arachidonic acid
AD	atopic dermatitis
ADCC	antibody-dependent cellular cytotoxicity
AEC	absolute eosinophil count
AHR	aryl hydrocarbon receptor
ANCA	anti-neutrophil cytoplasm antibody
APC	antigen-presenting cell
AT	adipose tissue
ATG	autophagy-related

BCG	Bacillus Calmette-Guérin
BP	bullous pemphigoid
C. burnetii	*Coxiella burnetii*
C_4	eoxin C_4
cAMP	cyclic adenosine monophosphate
CCL	C-C motif chemokine ligand
CCR	C-C chemokine receptor
CLC	Charcot-Leyden crystal protein
Clec4a4	C-type lectin domain family 4, member a4
CMP	common myeloid progenitor
COPD	chronic obstructive pulmonary disease
CRC	colorectal cancer
CXCL	C-X-C motif chemokine ligand
CXCR	C-X-C motif chemokine receptor
CysLT	cysteinyl leukotriene
CysLTR	cysteinyl leukotriene receptor
DAMP	damage-associated molecular pattern
DC	dendritic cell
DNA	deoxyribonucleic acid
ECM	extracellular matrix
ECP	eosinophil cationic protein
EDN	eosinophil-derived neurotoxin
EET	eosinophil extracellular trap
EGFR	epidermal growth factor receptor
EGPA	eosinophilic granulomatosis with polyangiitis
EM	eosinophilic myocarditis
EoE	eosinophilic esophagitis
EoP	eosinophil-lineage committed progenitor
EoPre	eosinophil precursor
EoSV	eosinophil sombrero vesicle
EPX	eosinophil peroxidase
ER	endoplasmic reticulum

FasL	Fas ligand
FDA	United States Food and Drug Administration
FeNO	fractional exhaled nitric oxide
FGF	fibroblast growth factor
fMLP	N-formyl-met-leu-phe
FPR	formyl peptide receptors
Gal-10	galectin-10
GI	gastrointestinal
GLUT	glucose transporter
GM-CSF	granulocyte-macrophage colony-stimulating factor
GM-CSFR	granulocyte-macrophage colony-stimulating factor receptor
GMP	granulocyte-macrophage progenitor
GPCR	G-protein-coupled receptor
GTP	guanosine triphosphate
HB-EGF-LBP	heparin-binding epidermal growth factor-like binding protein
HE	hypereosinophilia
HES	hypereosinophilic syndrome
HE_{US}	hypereosinophilia of undetermined significance
HSC	hematopoietic stem cell
IBD	inflammatory bowel disease
iEos	inflammatory eosinophil
IFN	interferon
Ig	immunoglobulin
IL	interleukin
ILC2	type 2 innate lymphoid cell
IL-1R	IL-1 receptor
IL-2R	IL-2 receptor
IL-3R	IL-3 receptor
IL-4R	IL-4 receptor
IL-5R	IL-5 receptor
IL-9R	IL-9 receptor
IL-10R	IL-10 receptor

IL-12R	IL-12 receptor
IL-13R	IL-13 receptor
IL-17R	IL-17 receptor
IL-23R	IL-23 receptor
IL-27R	IL-27 receptor
IRF	interferon regulatory factor
JAK	Janus kinase
LTB_4	leukotriene B_4
LTC_4	leukotriene C_4
LTD_4	leukotriene D_4
LXA_4	lipoxin A_4
LysoPS	lysophosphatidylserine
MAPK	mitogen-activated protein kinase
MBP	major basic protein
MC	mast cell
MetS	metabolic syndrome
MHC-II	major histocompatibility complex-II
MLKL	mixed lineage kinase-like protein
MMP	matrix metalloproteinase
mtDNA	mitochondrial DNA
mTORC1	mammalian target of rapamycin complex 1
NADPH	nicotinamide adenine dinucleotide phosphate
NGF	nerve growth factor
NK	natural killer cell
NMO	neuromyelitis optica
NOD	nucleotide-binding oligomerization domain-like
P2Y12R	P2Y12 receptor
PAF	platelet-activating factor
PAFR	platelet-activating factor receptor
PAMP	pathogen-associated molecular pattern
PAPP-A	pregnancy-associated plasma protein A
PD-L1	programmed death ligand 1

PDGF	platelet-derived growth factor
PDGFR	platelet-derived growth factor receptor
PG	prostaglandin
PI3K	phosphoinositide 3-kinase
PMD	piecemeal degranulation
proMBP	precursor of MBP
PRR	pattern recognition receptor
PVAT	perivascular adipose tissue
RAGE	receptor for advanced glycation endproduct
rEos	resident eosinophil
RIPK	receptor-interacting protein kinase
RNA	ribonucleic acid
RNase A	ribonuclease A
ROS	reactive oxygen species
RSV	respiratory syncytial virus
SAT	subcutaneous adipose tissue
SCF	stem cell factor
siglec	sialic acid-binding immunoglobulin-like lectin
SLE	systemic lupus erythematosus
SNAP23	synaptosome-associated protein 23
SNARE	soluble NSF attachment protein receptor
SP-D	surfactant protein-D
TATE	tumor-associated tissue eosinophilia
TCA	tricarboxylic acid
TF	transcription factor
TGF	transforming growth factor
Th	T helper
TLR	toll-like receptor
TME	tumor microenvironment
TNF	tumor necrosis factor
TNFR	tumor necrosis factor receptor
Treg	regulatory T cell

Trk-B	tropomyosin receptor kinase B
TSLP	thymic stromal lymphopoietin
TSLPR	thymic stromal lymphopoietin receptor
TXB_2	thromboxane B_2
TYK2	tyrosine kinase 2
VAMP	vesicle-associated membrane protein
VEGF	vascular endothelial growth factor
WBC	white blood cell
YB-1	Y-box-binding factor

Conflict of Interest

The authors declare no financial or commercial conflict of interest.

Author Contributions

Conceptualization: LG, SY, DS, HUS

Methodology: LG

Investigation: LG

Visualization: LG, TF, SY

Supervision: SY, DS, HUS

Writing – original draft: LG, TF

Writing – review & editing: LG, TF, SY, DS, HUS

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Tables

Table 1. Migration & activation factors of eosinophils

Factors	Potential cell sources of factors	Receptors expressed in eosinophils	Functional consequences in eosinophils	Ref.

Chemokines	CCL3/MIP1-α CCL5/RANTES CCL7/MCP-3 CCL8/MCP-2 CCL11/Eotaxin-1 CCL13/MCP-4 CCL22/MDC CCL24/Eotaxin-2 CCL26/Eotaxin-3 CXCL8/IL-8	- Airway smooth-muscle cells - Basophils - DCs - Endothelial cells - Epithelial cells - Eosinophils - Fibroblasts - Lymphocytes - Macrophages - Monocytes - Multiple tissues - Platelets - T cells - Tumor cells	CCR1 CCR3 CCR4 CCR5 CCR6 CCR8 CCR9 CXCR2 CXCR3 CXCR4	- Activation - Effector functions - Recruitment - Trafficking	12,94,96-110,286,288,327
Cytokines	GM-CSF IFN-γ IL-1α IL-2 IL-3 IL-4 IL-5 IL-9 IL-10 IL-12 IL-13 IL-16 IL-17 IL-23 IL-25/IL-17E IL-27 IL-33 TNF TSLP	- Adipocytes - B cells - Basophils - DCs - Endothelial cells - Eosinophils - Epithelial cells - Fibroblasts - Innate lymphoid cells - Macrophages - Monocytes - MCs - Neutrophils - NK cells - Platelets - T cells	GM-CSFR IFNGR IL-1R1 IL-2R IL-3R IL-4R IL-5R IL-9R IL-10R IL-12R IL-13R CD4 IL-17R IL-23R IL-17BR IL-27R ST2/IL-1RL1 TNFR TSLPR	- Activation - Differentiation & maturation - Gene & protein expression - Priming - Receptor expression - Survival - Trafficking	12,72,114,288,328-334
Other factors	Bacterial nucleic acids Bacterial proteins, lipoproteins and polysaccharides Fungal glucans Viral nucleic acids	- Bacteria - Fungi - Viruses	TLR1 TLR2 TLR3 TLR4 TLR5 TLR7 TLR8 TLR9 TLR10	- Activation	132,133,236,238
	Adenosine	- MCs	A_3	- Effector functions	9
	Histamine	- Basophils - MCs	H_4R	- Recruitment	9,12

	LL-37	- DCs - Epithelial cells - Lymphocytes - Macrophages - MCs - Monocytes - Neutrophils - NK cells	CXCR2 FPR-2 P2X7	- Release of inflammatory mediators	7,335
	LTC_4 LTD_4	- Basophils - MCs	CysLTR1 CysLTR2	- Effector functions - Recruitment - Trafficking	9,12
	LysoPS		P2Y10	- Effector functions	139

Table 2. Selection of eosinophilic diseases and the pathogenic role of eosinophils as target of treatment

Eosinophilic diseases	**Proposed role of eosinophils**	**Main treatment (with focus on eosinophil-targeting therapies)**	**Ref.**
Eosinophilic airway diseases			
Chronic obstructive pulmonary disease (COPD)	- Release of toxic granule proteins and pro-inflammatory mediators from eosinophils - Th1-mediated immune response	- Corticosteroids - HDAC2 activator (theophylline) in combination with corticosteroids - Long-acting muscarinic antagonist (LAMA) in combination with long-acting β2-agonist (LABA)	336,337
Chronic rhinosinusitis (with nasal polyps)	- EET formation - Release of toxic granule proteins and pro-inflammatory mediators from eosinophils - Th2-mediated immune response	- Anti-IgE antibody (omalizumab) - Anti-IL-4Rα antibody (dupilumab) - Anti-IL-5 antibody (mepolizumab) - Corticosteroids - Nasal steroids - Nasal douching - Surgery	131,338
Eosinophilic asthma	- EET formation - Release of toxic granule proteins and pro-inflammatory mediators from eosinophils - Th2-mediated immune response	- Anti-IgE antibody (omalizumab) - Anti-IL-4Rα antibody (dupilumab) - Anti-IL-5 antibody (mepolizumab, reslizumab) - Anti- IL-5Rα antibody (benralizumab)	130,336,338

		- Anti-TSLP antibody (tezepelumab, approved by FDA, not yet by EMA) - Corticosteroids - HDAC2 activator (theophylline) in combination with corticosteroids - Leukotriene receptor antagonist (montelukast)	
Eosinophilic dermatoses			
Allergic contact dermatitis	- EET formation in acute allergic contact dermatitis - Th1-mediated immune response	- Corticosteroids	45,130,131,339
Atopic dermatitis	- EET formation in acute atopic dermatitis - Release of toxic granule proteins, neuromediators and cytokines - Type 2 cytokines expression	- Anti-IL-4Rα antibody (dupilumab) - Anti-IL-13 antibody (tralokinumab, lebrikizumab) - Cyclosporin - JAK inhibitor (baricitinib, upadacitinib, abrocitinib) - Topical corticosteroids and topical calcineurin inhibitors	45,130,131,339,340
Bullous pemphigoid	- Contribution to edema formation through release of toxic granule protein release and production of leukotriene - Regulation of blister formation through EET formation - Tissue damage	- Anti-eotaxin-1 antibody (bertilimumab) - Anti-IL-4Rα antibody (dupilumab) - Anti-CD20 antibody (rituximab) - Corticosteroids	45,123,130,131,339,341
Eosinophilic dermatosis of hematologic malignancy (EDHM)	- Eosinophil accumulation and activation following IL-5 release from Th2 cells reactive to malignant B cells.	- Treatment of underlying disease - Anti-IL-4Rα antibody (dupilumab)	339,342
Parasitic infections	- EET formation - Host defense response	- Anti-helminthic drugs	131,339,343

	- Host tissue damage - Release of toxic granule proteins and pro-inflammatory mediators from eosinophils - ROS production		
Urticaria	- Contribution to edema and wheal formation - Mast cell stimulation through toxic granule protein release from eosinophils - Tissue factor, VEGF and leukotriene expression	- Anti-IL-5 antibody (mepolizumab, reslizumab) - Anti-siglec-8 antibody (antolimab) - Anti-IgE antibody (omalizumab)	45,339,344
Gastrointestinal disorders			
Eosinophilic colitis	- Peripheral blood eosinophilia - Presence of eosinophils in the stool - Selective accumulation of eosinophils in the in the colon	- Corticosteroids - Food elimination diet - Immunosuppressive agents	345-347
Eosinophilic enteritis	- Selective infiltration of eosinophils in the small intestine	*Unknown*	345
Eosinophilic esophagitis (EoE)	- EET formation - Esophageal subepithelial fibrosis and remodeling - Potential peripheral eosinophilia - Presence of intraepithelial eosinophils in proximal and distal esophagus - Th2-mediated immune response	- Anti-IL-4Rα antibody (dupilumab) - Corticosteroids - Esophageal dilation - Food elimination diet - Proton pump inhibitor	131,345-348
Eosinophilic gastritis	- Extracellular deposition of toxic granule protein - Selective infiltration of eosinophils in the stomach	- Corticosteroids - Food elimination diet - Immunosuppressive agents - Proton pump inhibitor	345-347

Eosinophilic gastroenteritis	- Extracellular deposition of toxic granule protein - Selective infiltration of eosinophils in the stomach and the small intestine	- Corticosteroids - Food elimination diet - Immunosuppressive agents - Proton pump inhibitor	345-349
Gastroesophageal reflux disease (GERD)	- Presence of intraepithelial eosinophils in the distal esophagus	- Histamine H2-receptor antagonists - Proton pump inhibitor	345
Inflammatory bowel disease (IBD)	- Extracellular deposition of toxic granule protein - Potential mucosal eosinophilia - Potential peripheral eosinophilia - Tissue damage	- Aminosalicylates - Antibiotics - Anti-Integrin antibody - Anti-TNF antibodies - Corticosteroids - Immunomodulators - JAK Inhibitors - Surgery	346,350,351
Other diseases			
Hypereosinophilic syndrome (HES)	- EET formation - Persistent peripheral blood hypereosinophilia (≥ 1'500 cells/mm3) - Potential tissue and organ damage Promotion of inflammation and tissue remodeling - Release of toxic granule proteins - Tissue hypereosinophilia	- Anti- IL-5Rα antibody (benralizumab) - Anti-IL-5 antibody (mepolizumab) - Cyclosporin - Corticosteroids - Hydroxyurea - IFN-α - Tyrosine kinase inhibitor (imatinib mesylate)	45,164-166,345,352

Table 3. Mediators and receptors expressed by eosinophils

A.	Proteins	Localization in eosinophils	Functions	Ref.
	CLC protein / Gal-10	*Unknown*	- B cells IgE production - ECP & EDN sequestration & vesicular transport - Granulogenesis - Neutrophilic inflammation - NLRP3 inflammasome activation - Type 2 sensitization	81,84,85,88,90,91,93
	ECP	- Secretory granules (matrix)	- Anti-bacterial, antiviral & anti-parasitic properties - Cytotoxicity against mammalian cells - EET formation	122-124,129,153,353
	EDN	- Secretory granules (matrix)	- Antiviral properties	126-128,170,354-356

			- Cytotoxicity against mammalian cells - DC activation & maturation - Neurotoxicity	
	EPX	- Secretory granules (matrix)	- Anti-parasitic properties - Cytotoxicity against mammalian cells - EET formation - Generation of oxidant species	135,141,143,144,357,358
	MBP	- Secretory granules (crystalline core)	- Anti-bacterial & anti-parasitic properties - Basophils and mast cells release of mediators - Cytotoxicity against mammalian cells - DC activation & maturation - EET formation	129,153,170,196,197,353
B.	**Chemokines**	**Localization in eosinophils**	**Functions**	**Ref.**
	CCL3/MIP1-α	*Unknown*	- Eosinophil activation & degranulation - Eosinophil recruitment - T cell recruitment	359
	CCL5/RANTES	- Secretory granules (matrix) - Secretory vesicles	- Eosinophil activation & degranulation - Eosinophil recruitment - T cell recruitment	95,328,359
	CCL11/Eotaxin	- Secretory granules	- Eosinophils, basophils, macrophages, DCs, & endothelial cells recruitment	95,360
	CCL13/MCP-4	- Secretory granules	- Basophil release of histamine - Eosinophil respiratory burst - Eosinophils, monocytes, basophils & T cells recruitment	95,361
	CCL17/TARC	*Unknown*	- Th2 cells recruitment	362
	CCL22/MDC	*Unknown*	- Eosinophil degranulation - Eosinophil recruitment - Th2 cells recruitment	362,363
	CCL23	*Unknown*	- DCs, monocytes & resting T cells recruitment - Inhibition of myeloid progenitor development	364
	CXCL1	- Secretory granules	- Neutrophil recruitment	365
	CXCL5	*Unknown*	- Neutrophil recruitment	169

	CXCL8/IL-8	*Unknown*	- T cells, basophils, & eosinophils recruitment	366
	CXCL9/MIG	*Unknown*	- T cells, NKs, & macrophages recruitment - Th1 cell polarization	362,367
	CXCL10/IP-10	*Unknown*	- T cells, NKs, & macrophages recruitment - Th1 cell polarization	362,367
	CXCL11/I-TAC	- Secretory granules (matrix)	- T cells, NKs, & macrophages recruitment - Th1 cell polarization	367
C.	**Cytokines**	**Localization in eosinophils**	**Functions**	**Ref.**
	APRIL	*Unknown*	- Plasma cell survival	174
	GM-CSF	- Secretory granules (crystalline core)	- Eosinophil degranulation - Eosinophil differentiation & maturation - Eosinophil superoxide production	20,328
	IFN-γ	- EoSVs - Secretory granules (matrix)	- Eosinophil activation & inhibition - Eosinophil degranulation - T cells recruitment	95,368,369
	IL-1α	*Unknown*	- Eosinophil antigen presentation	370
	IL-1β	*Unknown*	- Th17 cell polarization	371
	IL-2	- Secretory granules (crystalline core)	- Eosinophil recruitment - T cell recruitment	95,372
	IL-3	*Unknown*	- Eosinophil degranulation - Eosinophil differentiation & maturation - Eosinophil superoxide production - Protein synthesis	20,328,373
	IL-4	- Secretory granules (crystalline core) - Secretory vesicles	- B cell IgG4 & IgE production - Eosinophil differentiation - Macrophage polarization - Th2 cell polarization & expansion - Tissue homeostasis & repair	69,174,280,281, 374,375
	IL-5	- Secretory granules	- Eosinophil activation & degranulation - Eosinophil differentiation & maturation - Eosinophil superoxide production	20,328,376
	IL-6	- Secretory granules (matrix)	- Cofactor in B cell IgE production - Hematopoietic progenitor cells proliferation	95,171,175,377

			- NK cell activation - Plasma cell survival - T cell & B cell differentiation	
	IL-9	- Secretory granules - TfnRc^{+} endosome-like vesicles	- Mast cells & hematopoietic progenitors proliferation & differentiation - Th2 immune response	378,379
	IL-10	- Secretory granules	- B cell IgE production - Myeloid DCs proliferation - Th1 and Th2 immune response control	95,380,381
	IL-11	*Unknown*	- B cell differentiation - Inhibition of Th1 differentiation	382,383
	IL-12	- Secretory granules	- Inhibition of eosinophil differentiation - NK cell activation - Th1 differentiation	95,171,384
	IL-13	- Secretory granules	- B cell IgG4 and IgE production - Eosinophil recruitment - Macrophage polarization - Th2 immune response	69,374,379,385
	IL-16	*Unknown*	- CD4+ T cells, eosinophils, monocytes, & DCs recruitment	386
	IL-25/IL-17E	*Unknown*	- Eosinophil activation - Th2 differentiation by promoting eosinophil antigen uptake	387
	TNF	- Secretory granules (matrix)	- Eosinophil degranulation - Eosinophil superoxide production by eosinophils - Monocytes, T cells, neutrophils, & endothelial cells activation	95,328
D.	**Growth factors**	**Localization in eosinophils**	**Functions**	**Ref.**
	HB-EGF	*Unknown*	- Smooth muscle cells mitogenesis & recruitment	95
	NGF	*Unknown*	- Eosinophil degranulation - Eosinophil differentiation - Mast cells release of histamine - T cell & B cell proliferation & differentiation	95,388
	PDGF-B	*Unknown*	*Unknown*	95
	SCF	- Secretory granules	- Eosinophil activation & recruitment - HSCs regulation	389

			- Mast cell recruitment, proliferation, survival, & mediator release	
	TGF-α	- Secretory granules (matrix) - Secretory vesicles	- Growth & differentiation factor - Wound healing	390
	TGF-β	- Secretory granules (matrix)	- Inhibition of myeloid cell proliferation - Negative regulation of hematopoiesis - T cell & B cell maturation & differentiation	390
	VEGF	- Secretory granules	- Eosinophil degranulation - Eosinophil recruitment - Vascular endothelial cells growth factor	391
E.	**Lipid mediators**	**Localization in eosinophils**	**Functions**	**Ref.**
	5-lipoxygenase	- Lipid bodies	- Catalysis of arachidonic acid to leukotrienes	193
	15-lipoxygenase	- Lipid bodies	- DCs, T cells & mast cells recruitment - Eosinophilic inflammation	392
	Cyclooxygenase	- Lipid bodies	- Catalysis of arachidonic acid to prostaglandin	193
	LTC_4	- Lipid bodies - Nuclear envelope	- Eosinophil maturation & differentiation - Eosinophil recruitment, survival & secretion	194
F.	**Receptors**	**Localization in eosinophils**	**Functions**	**Ref.**
	2B4	- Plasma membrane	- Eosinophil activation & degranulation	393
	α4β1 (CD49d/29)	- Plasma membrane	- Eosinophil recruitment - Eosinophil rolling & adhesion - Interaction with VCAM-1 - Superoxide production	394
	α4β7 (CD49d/β7)	- Plasma membrane	- Eosinophil adhesion & rolling - Interaction with MAdCAM-1 & VCAM-1 - Regulator of eosinophil survival	394
	α6β1 (CD49f/29)	- Plasma membrane	- Eosinophil adhesion - Interaction with laminin	394
	αDβ2 (CD11d/18)	- Plasma membrane	- Eosinophil adhesion - Interaction with VCAM-1	394
	αLβ2 (CD11a/18)	- Plasma membrane	- Interaction with ICAMs	394
	αMβ2 (CD11b/18)	- Plasma membrane	- Eosinophil adhesion - Interaction with VCAM-1, ICAM-1 or albumin	394

αXβ2 (CD11c/18)	- Plasma membrane	- Interaction with VCAM-1, ICAM-1 or albumin	394
CD48	- Plasma membrane	- Crosstalk between eosinophils & themselves or other cell types - Low affinity ligand for CD2, high-affinity ligand for 2B4	393
CD58	- Plasma membrane	- Crosstalk between eosinophils & themselves or other cell types - High-affinity ligand for CD2	393
CD80	- Plasma membrane	- Antigen presentation	395
CD84	- Plasma membrane	- Crosstalk between eosinophils & themselves or other cell types - Interaction with SLAM	393
CD86	- Plasma membrane	- Antigen presentation	395
CD300a/Irp60	- Plasma membrane	- Negative regulator of eosinophil recruitment & IL-5-mediated eosinophil survival	396,397
CD300f	- Plasma membrane	- Regulation of IL-5-mediated eosinophil recruitment & activation	397
CysLT1R	- Granule membrane - Plasma membrane	- Eosinophil degranulation	248
CysLT2R	- Granule membrane - Plasma membrane	- Eosinophil degranulation	248
Dectin-1	- Intracellular compartments - Plasma membrane	- Pro-inflammatory cytokines generation	238
FcαR	- Plasma membrane	- Eosinophil activation - Interaction with adaptive immune responses	102
FcDR	- Plasma membrane	- Interaction with adaptive immune responses	102
FcγRIIB2 (CD32)	- Plasma membrane	- Eosinophil activation - Interaction with adaptive immune responses	102
FcμR	- Plasma membrane	- Interaction with adaptive immune responses	102
γδTCR	- Plasma membrane	- Cancer cytotoxicity - Eosinophil degranulation	398

		- Eosinophil ROS production	
LIR3/ILT5	- Plasma membrane	- Inhibitory functions	396
L-selectin	- Plasma membrane	- Eosinophil recruitment	399
MHC-II	- Plasma membrane	- Antigen presentation	395
NOD1	- Cytoplasm	- Eosinophil recruitment - Eosinophil surface adhesion molecules upregulation - Pro-inflammatory cytokines generation	238,400
NOD2	- Cytoplasm	- Eosinophil recruitment - Eosinophil surface adhesion molecules upregulation - Pro-inflammatory cytokines generation	238,400
NTB-A	- Plasma membrane	*Unknown*	393
P75/AIRM	- Plasma membrane	- Inhibitory functions	396
PAR1	- Intracellular - Plasma membrane	- cys-LT release - Eosinophil ROS production - Eosinophil shape change	401
PAR2	- Intracellular	- cys-LT release - Eosinophil shape change - ROS production by eosinophil	401
PD-L1	- Plasma membrane	- Inhibition of immune responses of ILC2, T cells, and myeloid cells	269
RAGE	- Plasma membrane	- Pro-inflammatory cytokines generation	238
siglec-3/CD-33	- Plasma membrane	*Unknown*	250
siglec-7	- Plasma membrane	- Downregulation of eosinophil activation	250,252
siglec-8	- Plasma membrane	- Eosinophil apoptosis	250,253
siglec-10	- Plasma membrane	*Unknown*	250
SNAP23	- Cytoplasm - Plasma membrane	- Fusion of granule or vesicles with plasma membrane	255
Syntaxin-4	- Plasma membrane	- Fusion of granule or vesicles with plasma membrane	255
Syntaxin-17	- EoSVs - Secretory granules	- Fusion of granule or vesicles with plasma membrane	254

		- Transport of granule-derived cargos	
TLR1	- Plasma membrane	- Eosinophil activation	238,239
TLR2	- Intracellular compartments - Plasma membrane	- Eosinophil activation & degranulation - Eosinophil release of IL-1β, IL-6, IL-8 & Gro-α - Eosinophil surface adhesion molecules upregulation	238,239
TLR3	- Endosomes	- Type I IFN transcription	238
TLR4	- Intracellular compartments - Plasma membrane	- Eosinophil activation	238,239
TLR5	- Intracellular compartments - Plasma membrane	- Eosinophil activation & degranulation - Eosinophil release of IL-1β, IL-6, IL-8 & Gro-α - Eosinophil surface adhesion molecules upregulation	238,239
TRL6	- Intracellular compartments - Plasma membrane	- Eosinophil activation	238,239
TLR7	- Endosomes	- Eosinophil activation & degranulation - Eosinophil release of IL-1β, IL-6, IL-8, Gro-α & superoxide - Eosinophil surface adhesion molecules upregulation - IFN production	238,239
TLR9	- Endosomes	- Eosinophil activation - IFN production	238,239
TLR10	- Endosomes	*Unknown*	238,239
VAMP-2	- Secretory vesicles	- Fusion of granule or vesicles with plasma membrane	255
VAMP-7	- Secretory granules - Small secretory vesicles	- Fusion of granule or vesicles with plasma membrane	255
VAMP-8	- Secretory granules - Small secretory vesicles	- Fusion of granule or vesicles with plasma membrane	255

Figure legends

Figure 1. The dual role of eosinophils in cancer. Eosinophil control tumor progression through the release of cytotoxic mediators, recruitment of cytotoxic $CD8^+$ T cells, polarization of macrophages to the anti-tumorigenic M1 phenotype and vascular healing. Conversely, eosinophils act as modulators of the tumor microenvironment by the recruitment of regulatory T cells and polarization of macrophages to pro-tumorigenic M2 phenotype. Additionally, eosinophils mediate tumor growth by facilitating metastasis formation, tumor migration, angiogenesis and wound healing. Abbreviations: CCL, C-C motif chemokine ligand. CXCL, C-X-C motif chemokine ligand. ECP, eosinophil cationic protein. EDN, eosinophil-derived neurotoxin. EPX, eosinophil peroxidase. FGF, fibroblast growth factor. IL, interleukin. M1, classically-activated macrophages. M2, alternatively-activated macrophages. MBP, major basic protein. MMP, matrix metalloproteinase. PDGF, platelet-derived growth factor. TNF-α, tumor necrosis factor α. T reg, regulatory T cells. VEGF, vascular endothelial growth factor.

Figure 2. Schematic representation of eosinophil-mediated immunoregulation. Eosinophils modulate distinct immune responses through the release of various pre-formed immunoregulatory factors such as granule proteins, cytokines, chemokines, growth factors, and lipid mediators. Abbreviations: APRIL, a proliferation-inducing ligand. BS, basophils. CCL, C-C motif chemokine ligand. CXCL, C-X-C motif chemokine ligand. DC, dendritic cells. EDN, eosinophil-derived neurotoxin. Eos, eosinophils. GM-CSF, granulocyte-macrophage colony-stimulating factor. IFN, interferon. IL, interleukin. LTC4, leukotriene C4. MØ, macrophages. MBP, major basic protein. MC, mast cells. MO, monocytes. NEU, neutrophils. NGF, nerve growth factor. NK, natural killer cells. PC, plasma cells. SCF, stem cell factor. TGF, transforming growth factor. TNF-α, tumor necrosis factor α. VEGF, vascular endothelial growth factor.

Figure 3. Functions of eosinophils. Eosinophils exert a wide range of functions. The cytotoxic functions of eosinophils comprise host protection against diverse pathogens and involvement in pathophysiologic conditions like allergy and autoimmune diseases, as well as cancer. Eosinophils take part in immunoregulation by modulating the functions of lymphocytes, dendritic cells, neutrophils, mast cells, and natural killer cells, as well as by participating in antigen presentation. Additionally, eosinophils perform homeostatic functions in a variety of tissues and play a role in wound healing and tissue remodeling. Abbreviations: AT, adipose tissue. BS, basophils. DC, dendritic cells. Eos, eosinophils. GI tract, gastrointestinal tract. MØ, macrophages. MC, mast cells. MO, monocytes. NEU, neutrophils. NK, natural killer cells. PC, plasma cells.

Figure 4. Schematic representation of eosinophil metabolism. Eosinophils use glycolysis as their main energy pathway, along with TCA cycle and mitochondrial oxidative phosphorylation. Glucose uptake is mediated by GLUTs, intracellular Ca^{2+} and MAPK pathway. ROS production through mitochondrial respiration activates ERK1/2 and caspase-3. Abbreviations: ATP, adenosine triphosphate. Acetyl-CoA, acetyl-coenzyme A. e^-, electron. ERK, extracellular-signal-regulated kinase. ETC, electron transfer chain. GLUT, glucose transporter. MAPK, mitogen-activated protein kinase. ROS, reactive oxygen species. TCA cycle, tricarboxylic acid cycle.

Figure 5. Schematic representation of eosinopoiesis. A Eosinophils are generated in the bone marrow under the influence of a set of distinct transcription factors. The expression of FOG-1 in HSCs, CMP, and GMP suppresses myeloid cell development. Subsequently, C/EBPα regulates GATA-1 expression which in combination with IRF8, GATA-2, and Xbp-1 induces eosinophil lineage commitment. Mouse EoPs arise from GMP or under the influence of IL-33 from GMP-like progenitors, whereas human CMP evolves into EoPs independently of GMP generation. Relevant markers expressed by the different eosinophil precursors are shown. **B** Terminal differentiation of eosinophils from EoPs is mediated by the transcription

factors PU.1, GATA-1, Xbp-1, and C/EBPε. Eosinophil differentiation is characterized by the generation of granules. Primary, larger granules are mainly found in promyelocytes, whereas secondary granules are the predominant granules in mature eosinophils. Abbreviations: C/EBP, CCAT/enhancer-binding protein. CMP, common myeloid progenitor. EoP, eosinophil progenitor. Eos, eosinophil. FOG-1, friend of GATA-1. GMP, granulocyte-macrophage progenitor. HSC, hematopoietic stem cell. IL, interleukin. MB, myeloblast. MC, myelocyte. IRF, interferon regulatory factor. pMC, promyelocyte.

Figure 6. Schematic overview of diverse publications describing distinct eosinophil subpopulations. Diverse reports highlighted the heterogeneity of eosinophils in human and mouse systems over the last decades. The subpopulations were defined based on different cytokine expression patterns, differential surface protein expression, distinct phenotypes, and functions, as well as based on homeostatic or inflammatory conditions. Differentially expressed genes are shown in italic, while differentially expressed proteins are written in regular style. In this figure, "dendritic" extensions on eosinophil are used to represent the subset of villus intestinal resident CD11c^{high}-eosinophils reported by Xenakis *et al.*[267]. Abbreviations: AHR, aryl hydrocarbon receptor. CCL, C-C motif chemokine ligand. CCR, C-C chemokine receptor. Clec4a4, C-type lectin domain family 4, member a4. CXCL, C-X-C motif chemokine ligand. CXCR, CXC chemokine receptor. Eos, eosinophils. IFN, interferon. IL, interleukin. MHC, major histocompatibility complex. MMP, matrix metalloproteinase. PD-L1, programmed death-ligand 1. Siglec, sialic acid-binding immunoglobulin-like lectin. TGF, transforming growth factor. THBS, thrombospondin. TLR, toll-like receptor. TNF-α, tumor necrosis factor α. TSLP, thymic stromal lymphopoietin. TSLPR, TSLP receptor.

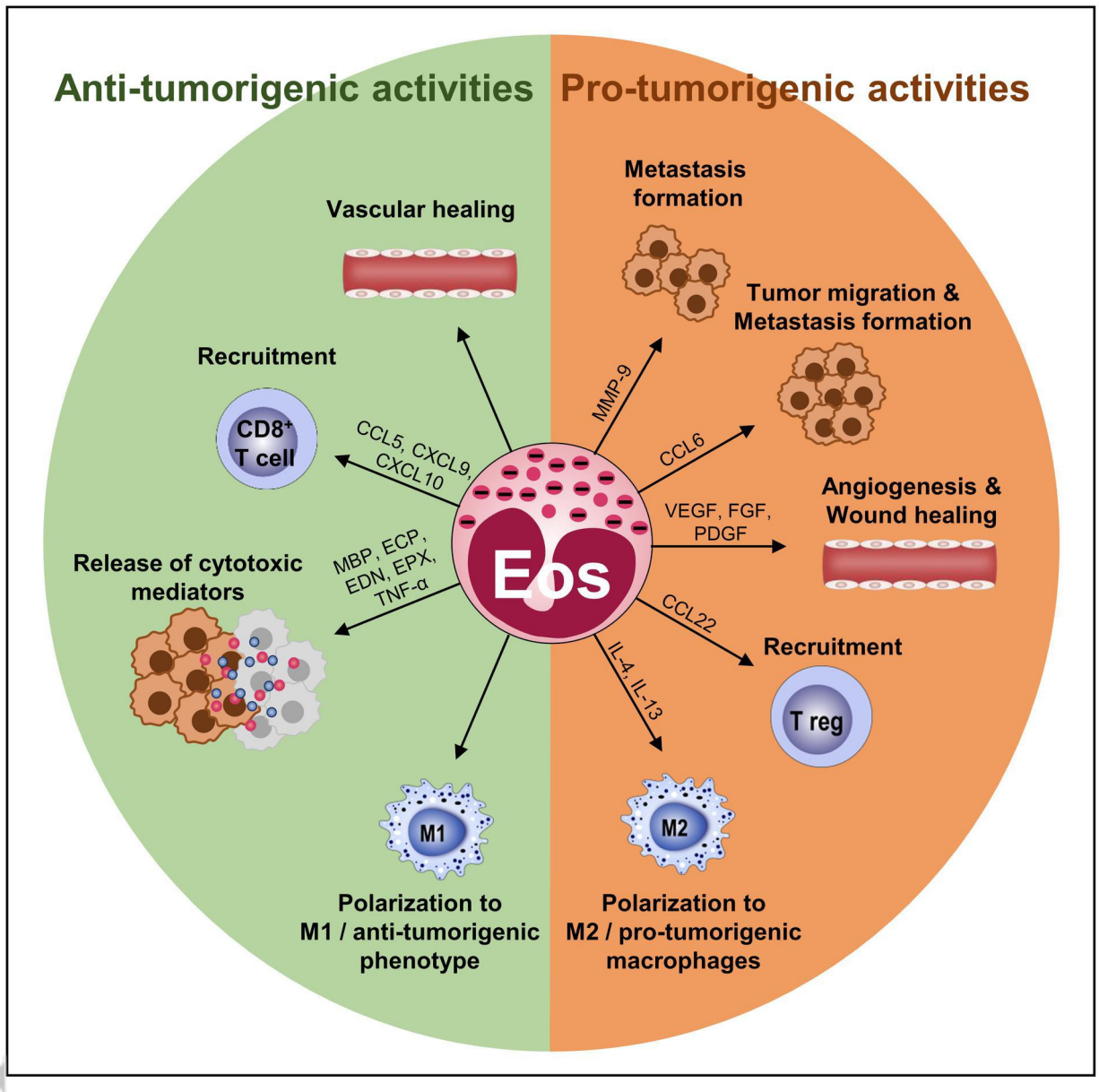

Figure 1_Gigon et al.

Figure_1.jpg

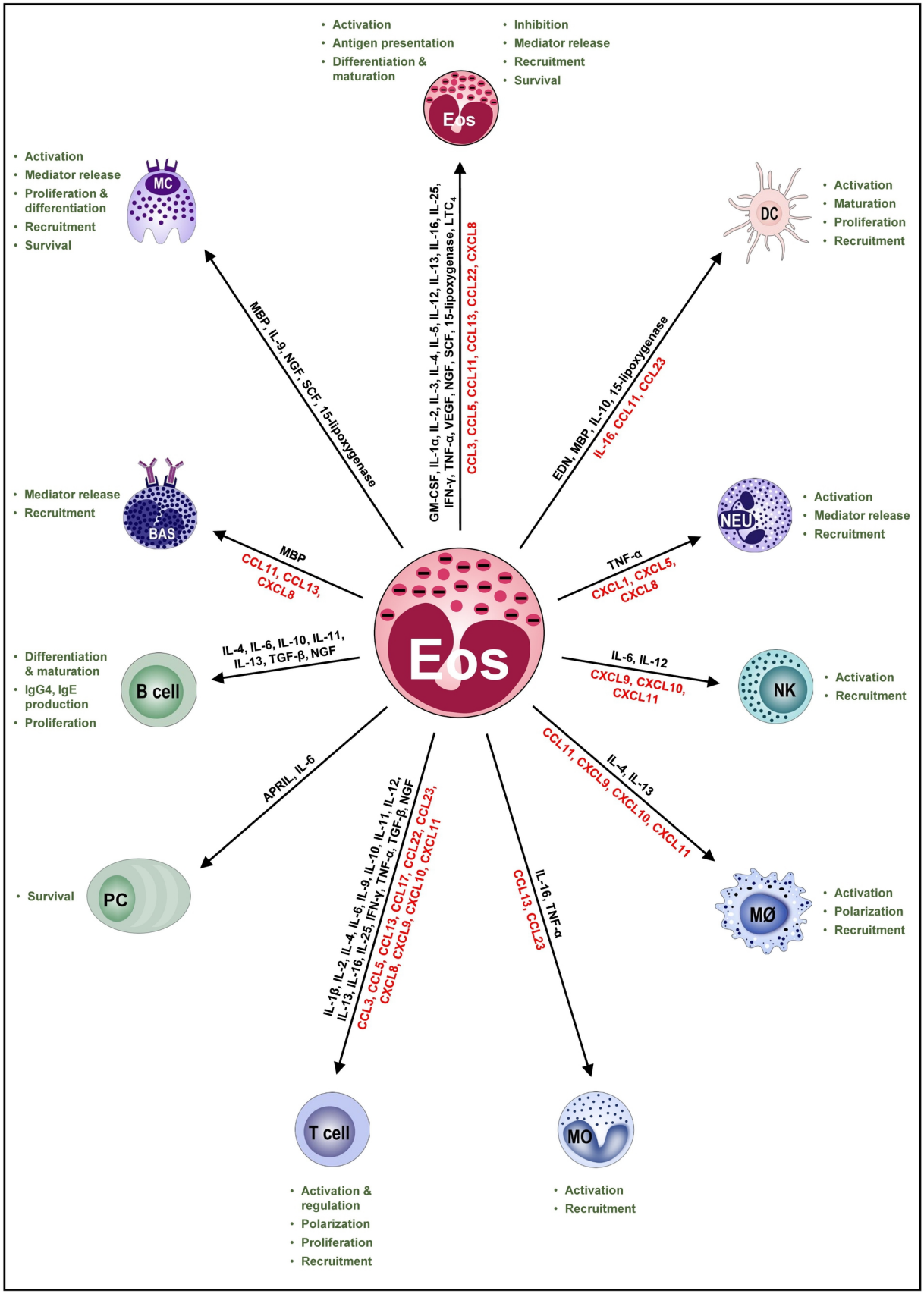

Figure 2_Gigon et al.

Figure_2.jpg

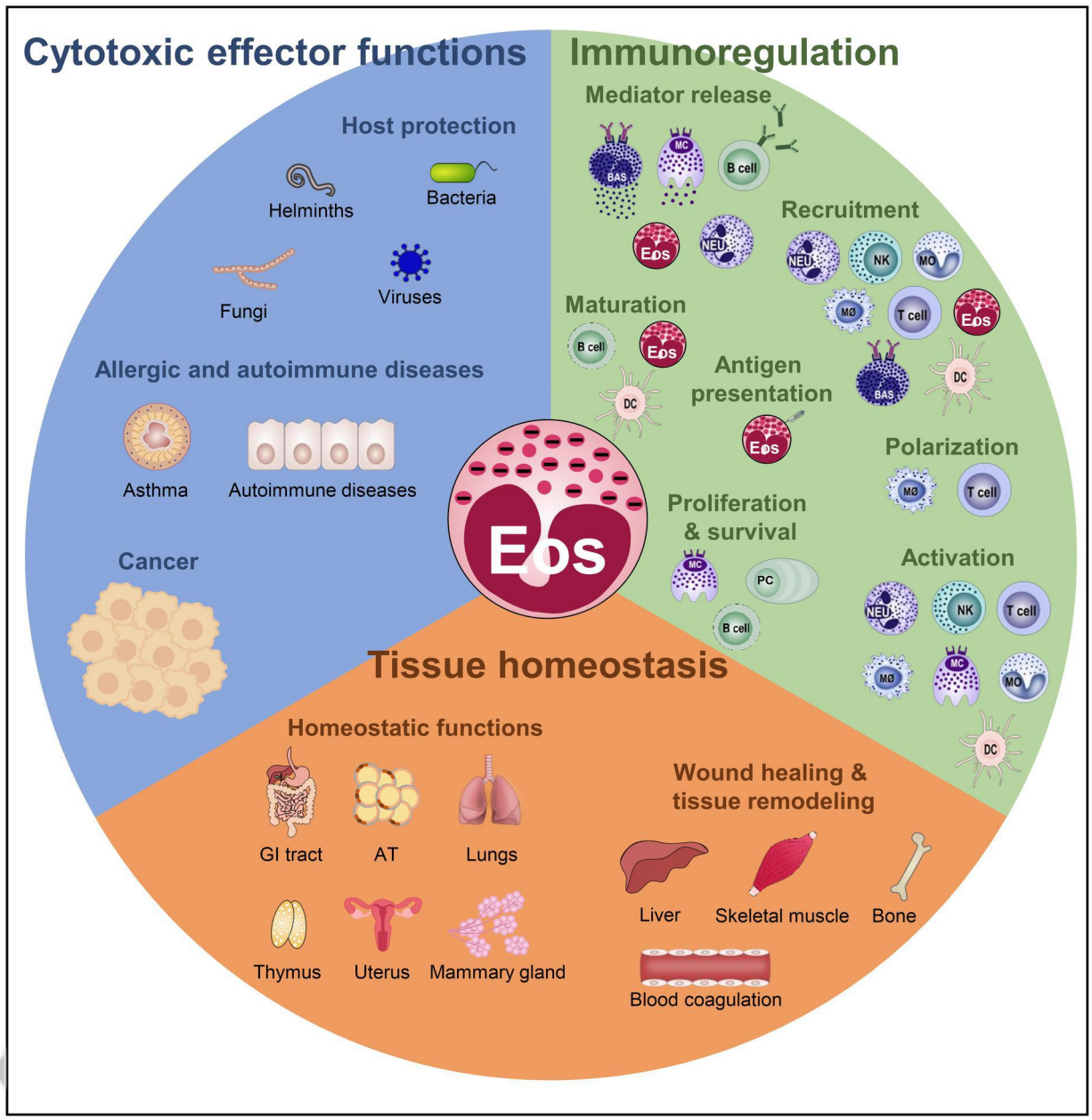

Figure 3_Gigon et al.

Figure_3.jpg

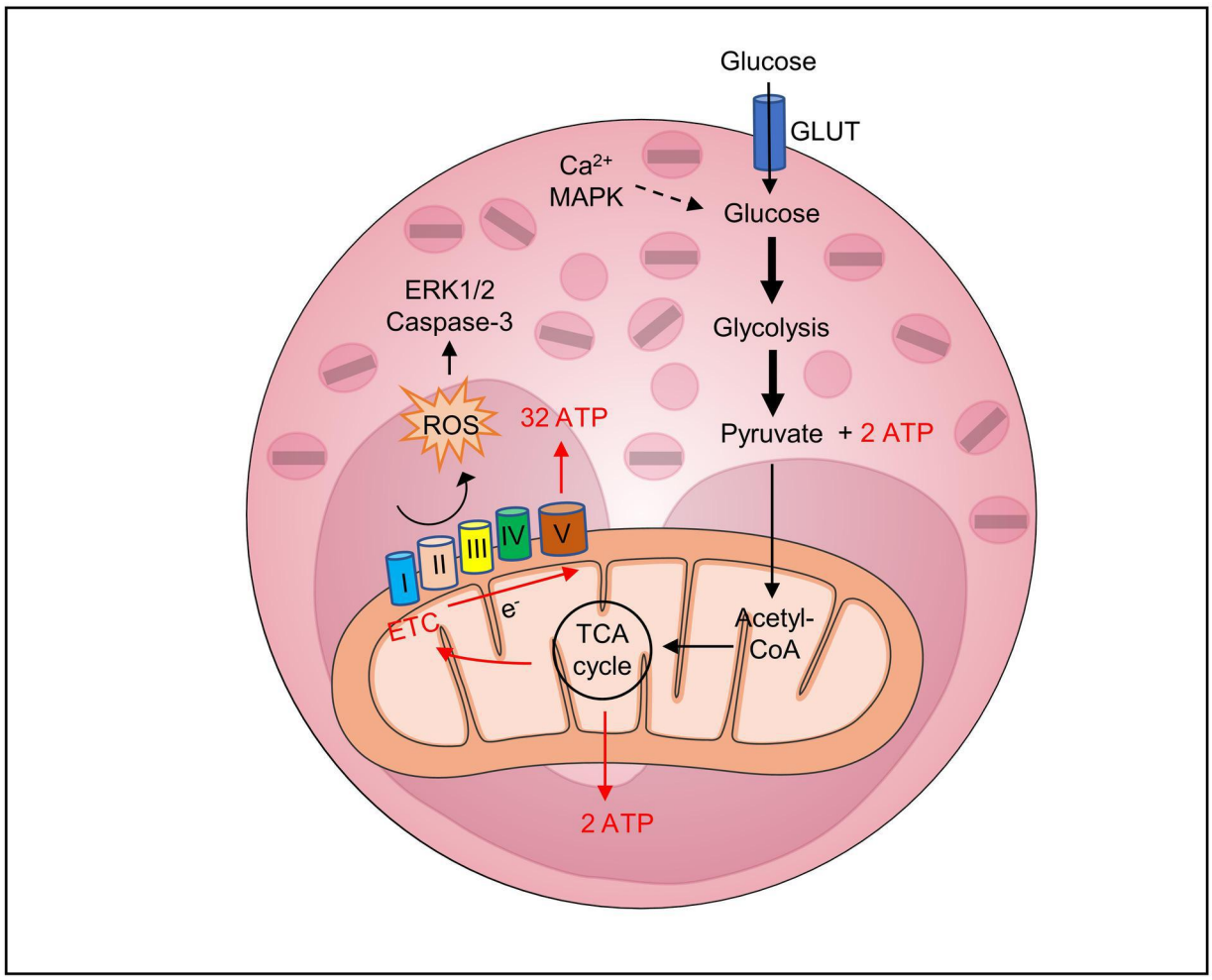

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Figure_4.jpg

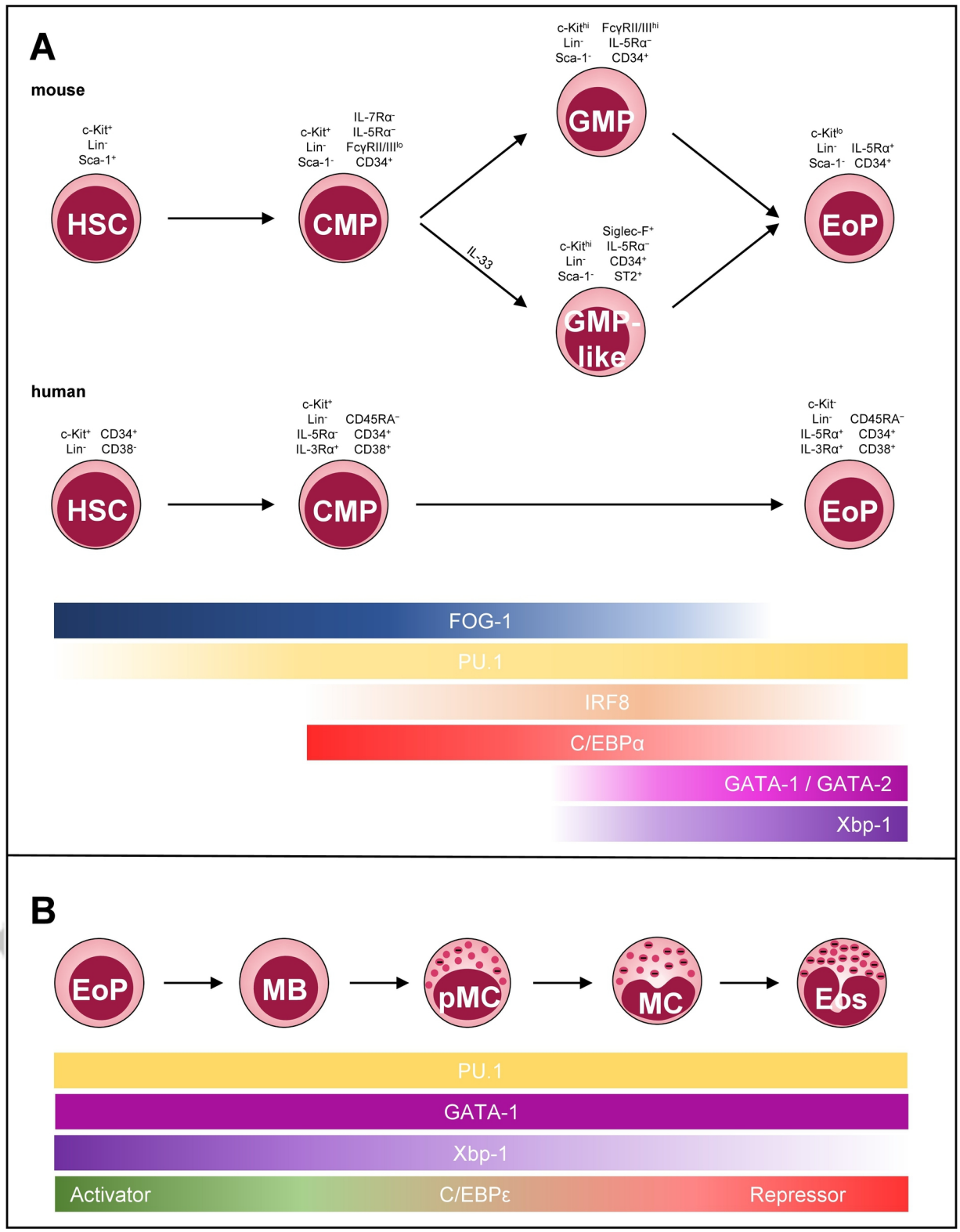

Figure 5_Gigon et al.

Figure_5.jpg

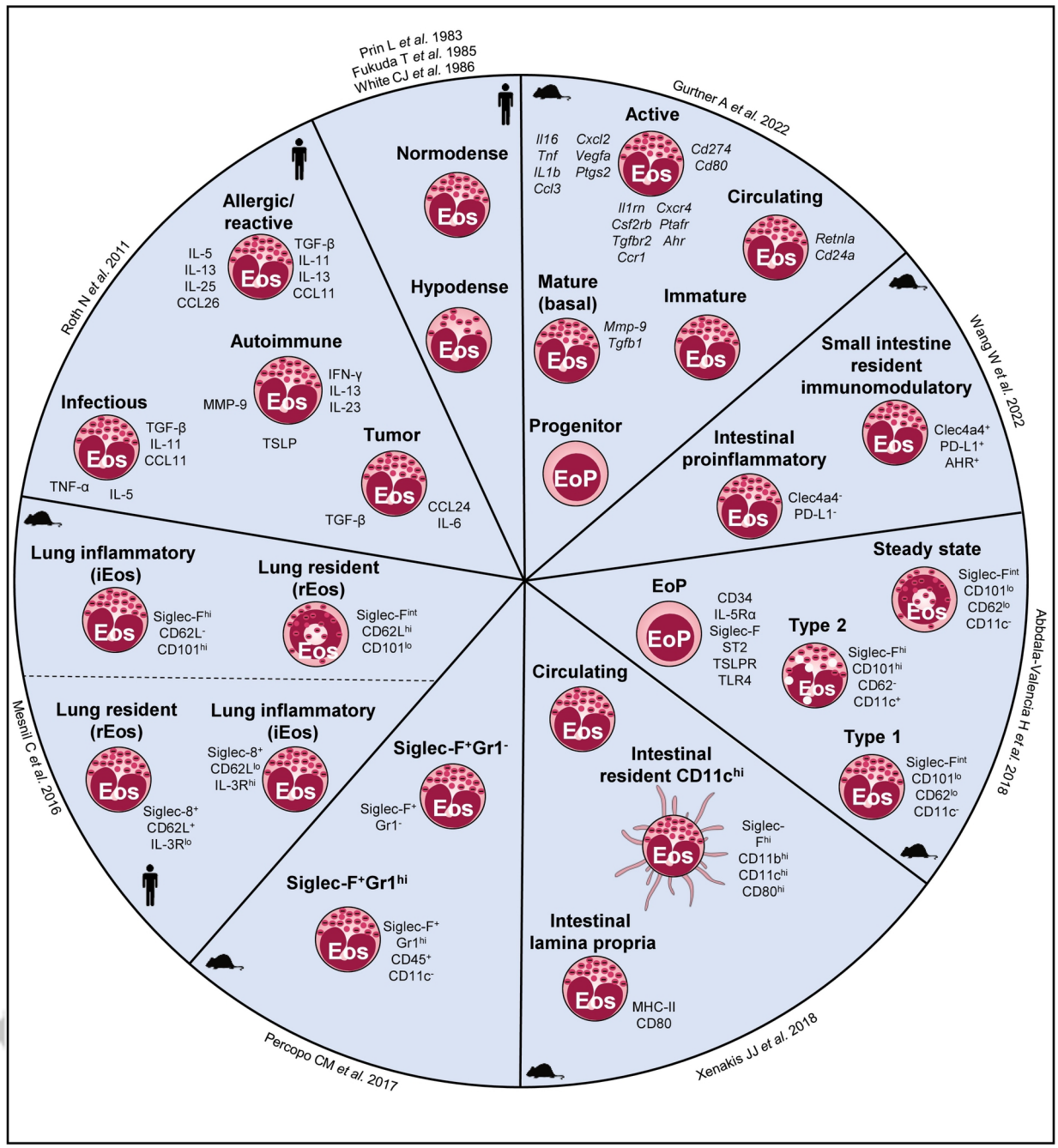

Figure 6_Gigon et al.

Figure_6_revised.jpg