Immunomodulation by basophilic granulocytes: fiction and facts

Basophilic orchestration of allergic inflammatory responses

Writing assignment literature review

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Abstract

Basophils are blood-circulating leukocytes that make up less than 1% of peripheral immune cells. For a long time, basophils have been overshadowed by mast cells, but despite several phenotypic and functional similarities, they are distinct cells with distinct effector and regulatory functions in a variety of diseases. Basophils are recruited to tissues where they can be activated by IgE-dependent and IgE-independent mechanisms to secrete a broad range of preformed or *de novo* synthesized inflammatory molecules. These molecules include histamine, platelet-activating factor (PAF), leukotriene C_4 (LTC_4), various chemokines as well as the type 2 cytokines interleukin (IL)-4 and IL-13. By releasing these proteins and mediators, basophils contribute to host immunity against parasitic infections. Basophils are associated with autoimmune disorders and several malignancies, although their most prominent role is in allergic inflammatory responses, where they can act as effector cells in the early-phase response and regulatory cells in the late-phase response. By secreting cytokines and chemokines, basophils orchestrate the recruitment of other leukocytes to sites of inflammation, facilitating (late-phase) allergic responses. This review describes the fiction and facts of different basophil properties in the context of immunomodulation in allergic responses, and highlights potential basophil-targeting strategies. Further research on these enigmatic cells and the extent of their contribution to allergy and immunity will enable us to better understand and utilize these cells to their full potential.

Chapter 1 – Introducing basophils

Basophilic granulocytes (basophils) are the least abundant leukocytes in blood. Basophils were first discovered and described by Paul Ehrlich in 1879 (1). Due to their low abundance and phenotypical similarities to mast cells they were long believed to be merely blood-circulating mast cell precursors and were termed blood mast cells (2). Indeed, basophils and mast cells are alike in many aspects, most notably their granular phenotype and the expression of the high-affinity IgE receptor (FcεRI) on the cell surface. IgE-mediated activation of basophils and mast cells results in secretion of both preformed and *de novo* synthesized inflammatory mediators (3). Basophil and mast cell mediators include histamine, leukotrienes, cytokines and chemokines (4). Despite these similarities basophils and mast cells are distinct cells, with their own functions in health and disease.

After their initial discovery in 1879, basophils remained an overlooked and understudied cell type for many decades. This changed around the 1950s, when mast cells and basophils were linked to hypersensitivity (2). Further milestones in the unraveling of basophil biology and functions include the discovery of histamine and leukotriene release from activated basophils, IgE-mediated basophil activation and the discovery of interleukin (IL)-4 and IL-13 as key basophil cytokines (5,6). The development of new methods for the analysis of basophil activation and degranulation has contributed considerably to the current understanding of these cells as sentinels in host defense with a broad range of effector functions. Now, more than 140 years after the discovery of basophils, we know that these cells are associated with multiple inflammatory processes and disorders. By the release of inflammatory mediators, especially cytokines and chemokines, basophils have the ability to initiate and modulate immune responses. In this report, the fiction and facts of the wide array of basophil properties in the context of immune modulation, as well as a potential target for treatment, are introduced and discussed. The focus will be on basophils in allergic inflammatory disorders.

1.1 Basophil properties

Basophils are granulocytes of 5-8 µm in diameter with an indented or segmented nucleus (Figure 1). They circulate in peripheral blood, making up less than 1% of peripheral blood-circulating leukocytes (4). The lifespan of murine basophils is approximately 60 hours under homeostatic conditions, making them much more short-lived than mast cells, for which this can be several months (3). Together with other granulocytes, basophils can be among the first responders of the innate immune system by releasing the contents of their cytosolic granules. Basophilic granules contain a plethora of preformed inflammatory mediators. Different surface receptors allow basophils to respond to signals and interact with their environment, influencing their development and effector functions. An overview of a selection of basophil surface receptors is presented in Figure 1.

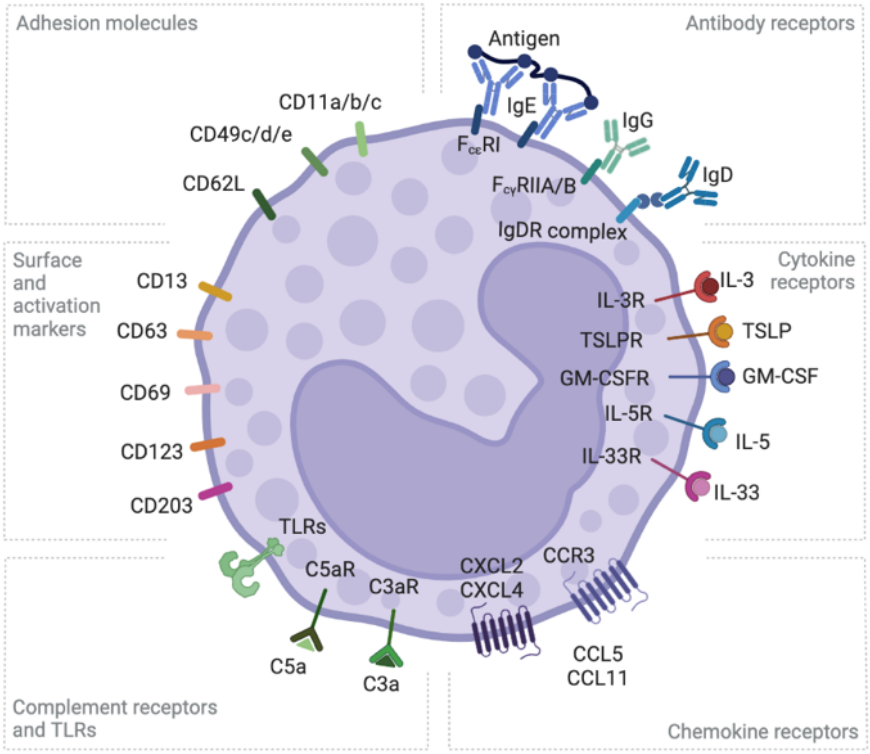

FIGURE 1. A selection of basophil surface molecules and receptors. Basophil surface molecules can be divided into several different categories: antibody receptors, cytokine and chemokine receptors, receptors for complement and Toll-Like receptors, markers of activation, and adhesion molecules. FcεRI, high-affinity IgE receptor; Ig(E/G/D), immunoglobulin; TSLP, thymic stromal lymphopoietin; GM-CSF, granulocyte-macrophage colony stimulating factor; CCL chemokine (C-C motif) ligand; CCR, C-C chemokine receptor; CXCL (C-X-C motif) ligand; TLR, toll-like receptor; CD, cluster of differentiation; CD62L, CD62 ligand. Figure made with BioRender.

1.2 Development and maturation

It is commonly believed that hematopoiesis of murine basophils is developmentally similar to that of mast cells, both originating from $CD34^+$ hematopoietic progenitor cells in the bone marrow. Hematopoietic stem cell-derived granulocyte-monocyte progenitors (GMP) develop into bipotent pre-basophil and mast cell progenitors (pre-BMP) and basophil-mast cell progenitors (BMCP) (7,8). In the bone marrow, pre-BMP develop into to unipotent basophil progenitors (BaP) giving rise to fully mature basophils that are released into circulation (7). Murine basophil development is regulated by the timed expression of different transcription factors, most notably CCAAT/enhancer binding protein-α (C/EBPα) and GATA-binding protein-2 (GATA-2) (9). Subsequent upregulation of C/EBPα induces differentiation of BMCP into BaP, which are characterized by high levels of C/EBPα and GATA-2. Similarly, C/EBPα expression is an important determinant of basophil lineage as opposed to mast cell lineage fate decision from pre-BMP in the bone marrow (9).

Human basophil development is not as well-characterized. Instead of similarities to mast cell development, human basophil development is more closely associated with eosinophil development and there may be a common eosinophil-basophil progenitor cell (10). Recent studies employing single-cell transcriptomics and fate assays suggest that the progenitors in mast cell and basophil hematopoiesis may be more heterogeneous than previously assumed. Indeed, basophil appear to be more closely related to megakaryocyte and erythroid lineages than granulocyte lineages (11). Fully elucidating the hematopoietic processes in human basophil development is a topic of ongoing research.

Unlike for mast cells, maturation of basophils occurs in the bone marrow, after which they are released into circulation. Exposure to the T cell-derived cytokine IL-3 is essential for development and maturation (12). In the absence of IL-3, the cytokine thymic stromal lymphopoietin (TSLP) is sufficient to induce basophil survival. TSLP-elicited and IL-3-elicited basophils appear represent two distinct types or activation modes of basophils, as demonstrated by distinct phenotypes, functions and transcriptional profiles (13). TSLP-elicited basophils are characterized by a smaller degree of degranulation and different surface receptor expression, indicating that they can respond differently to environmental stimuli (13).

1.3 Migration to tissues

Basophils circulate in peripheral blood and in order to exert effector functions locally in tissues, they have to migrate and cross the endothelial cell layer and basement membrane. Basophils constitutively express a series of cell adhesion molecules including integrins, selectins and adhesion molecules belonging to the immunoglobulin family (14). L-selectin (CD62L) is involved in basophil endothelial rolling and is downregulated upon activation and tissue entry. Exposure to IL-3 rapidly increases the expression of the β_2 integrin CD11b on human basophils, further increasing adhesion to vascular endothelium (15). The integrin very late activation antigen-4 (VLA-4; CD49d) allows basophils to adhere to endothelial vascular cell adhesion molecule-1 (VCAM-1), mediating transendothelial migration (14). Basophil chemotaxis is largely mediated by the C-C chemokine receptor type 3 (CCR3), which is constitutively expressed by basophils (16). The CCR3-ligands eotaxin (CCL11), regulated upon activation normal T cells expressed and secreted (RANTES; CCL5) and monocyte chemotactic protein (MCP) can induce chemotaxis of human blood basophils *in vitro* (16). Other inducers of basophil chemotactic activity include granulocyte-macrophage colony-stimulating factor (GM-CSF), complement C5a, IL-5 and to a smaller degree IL-8 (17).

1.4 Basophil activation

Activation of basophils can be mediated by a wide range of signals and molecules, including immunoglobulins, cytokines, chemokines, complement factors and bacteria-derived products. Some stimuli are sufficient to activate basophils on their own, while others require priming with an additional stimulus to elicit basophil responses.

1.4.1. Immunoglobulins

IgE – The classical activation mechanism of basophils is IgE-mediated activation. The high-affinity IgE receptor FcεRI is expressed by both murine and human basophils. FcεRI expression on basophils correlates with serum IgE concentrations, with higher IgE levels upregulating FcεRI expression (4). Cross-linking of two surface-bound IgE molecules by multivalent antigens induces basophil activation, resulting in Ca^{2+}-dependent release of preformed mediators stored in cytosolic granules and *de novo* synthesis of inflammatory mediators including cytokines and chemokines (18). IgE-mediated basophil activation and subsequent degranulation is accompanied by the translocation of granule membrane proteins to the basophil cell surface (19). The most important and useful activation markers for basophils are CD63 and CD203c, both of which are upregulated by IgE-mediated basophil activation (20).

IgG – Human basophils express both the activating FcγR-IIA (CD32a) and the inhibitory FcγR-IIB (CD32b) low affinity receptors for IgG (21). Upon IgG binding, the inhibitory signals from FcγR-IIB are dominant, preventing basophil activation (22). Engagement of both FcγR and FcεRI results in FcγR-IIB-dependent inhibition of IgE-mediated activation, with a further dampening effect in the presence of IL-3 (22). Rather than an activator, FcγR-IIB is thus a regulator of basophil activation.

IgD – The role of IgD in basophil activation is puzzling. Basophils can be activated by IgD despite the absence of an IgD Fc-receptor. Instead, IgD binds to basophils through a receptor complex consisting of galectin 9 and CD44 (23). Binding of antigen to basophil-bound IgD results in the release of type 2 pro-inflammatory cytokines, chemokines and antimicrobial peptides. Furthermore, antigen binding to basophil-IgD promotes B cell survival and enhances production of IgE and IgG1 by B cells, while suppressing antigen-IgE-mediated degranulation of basophils and mast cells (23,24). The latter may contribute to mucosal homeostasis and clearance of environmental antigens.

IgA – A limited number of studies have shown basophil activation by secretory IgA (sIgA), resulting in histamine release and leukotriene C_4 (LTC_4) production. Basophil activation by sIgA was dependent on priming with IL-3, IL-5 or GM-CSF (25). More recent work however, suggests allergen-specific IgA can suppress IgE-mediated basophil activation (26). The exact connections between IgA and basophils remain unclear and further study is needed.

1.4.2. Cytokines and chemokines

IL-3 – The T cell-derived cytokine IL-3 is a key player in basophil development and activation, affecting essentially all basophil effector functions. IL-3 stimulation alone does not strongly induce the release of histamine or IL-4, but instead IL-3 primes basophils for increased secretion of inflammatory mediators upon IgE-mediated activation (5,27). Not only IgE-mediated activation can be enhanced, other activation modes, for example complement-induced activation, are also dependent on IL-3 priming (28). Basophils themselves are also a source of IL-3, enabling autocrine priming for enhanced activity (29). While this cytokine is strongly associated with all basophil functions, experiments with IL-3-deficient mice have indicated that IL-3 is not crucial for basophil survival, but that basophil numbers and parasite expulsion are somewhat decreased in the absence of IL-3 (30).

TSLP – In the absence of IL-3, TSLP – expressed by epithelial cells – can promote basophilia and mediator release in mouse models (13). Conversely, TSLP can synergize with IL-3 to increase mediator release. TSLP has been shown to increase CCR3 expression on human basophils, rendering them increasingly sensitive to eotaxin-mediated migration from bone marrow into peripheral tissues (31).

IL-5 – IL-5 can contribute to basophil degranulation in conjunction with other activating molecules such as platelet-activating factor (PAF) or IL-8 (32). While IL-5 may induce LTC_4 secretion by itself, histamine release only occurs in conjunction with agonists that induce cytosolic Ca^{2+} (e.g. PAF or IL-8). The complementary signal of increased cytosolic Ca^{2+} appears to be essential for basophil degranulation mediated by IL-5, but also IL-3 and GM-CSF (32).

IL-33 – Similarly to IL-3, IL-33 – an epithelial alarmin – can promote cytokine secretion from human basophils in conjunction with IgE-mediated activation (33). IgE-independent stimulation of basophils with IL-33 can induce secretion of various cytokines, including IL-4, IL-5, IL-6, IL-8 and IL-13 (34).

IL-18 – IL-18 is another cytokine that can induce FcεRI-independent basophil activation. In response to IL-18, IL-3-primed basophils can secrete type 2 cytokines and histamine (35). Interestingly, IL-18 and IL-3 induce strong IL-4 production in basophils, which may be important in early type 2 immune responses (36).

GM-CSF – Granulocyte macrophage colony stimulating factor (GM-CSF) has been shown to contribute to basophil activation in combination with other modes of activation, such as IL-3, complement factors and IgE-mediated activation (37). Similarly to IL-5, GM-CSF on its own does not induce histamine release. Instead, a complementary signal that causes cytosolic Ca^{2+} release is required (32).

1.4.3 Complement

The anaphylatoxins C3a and C5a have been reported to activate basophils (38). Pre-incubation with IL-3 or GM-CSF renders basophils highly responsive to C3a and C5a, resulting in rapid histamine release and LTC_4 production. In contrast to C3a, C5a by itself is sufficient to induce histamine release. However, for LTC_4 secretion, IL-3-priming is required (38).

1.4.4 Toll like receptor agonists

The expression of various Toll Like Receptors (TLRs) allows basophils to recognize pathogens from microbes. TLR2 ligands induce secretion of cytokines, but not histamine or lipid mediators through both IgE-dependent and IgE-independent activation mechanisms (39). Similarly TLR4 may play a role in basophil activation and responses in allergic reactions (40).

1.5 Release of inflammatory mediators

Activation of basophils results in secretion of a plethora of immunomodulatory mediators. This occurs through degranulation of cytosolic granules containing preformed inflammatory mediators. Additionally, *de novo* synthesized mediators may be released, such as LTC_4, PAF, IL-4, IL-13, TSLP, chemokines and antimicrobial peptides. Basophil proteins and mediators are involved in initiation of type 2 inflammatory responses and in the acute and late-phase immune response. This section describes various prominent immunomodulatory basophil mediators (Table 1).

1.5.1. Histamine

Both basophils and mast cells are sources of histamine, although basophilic granules contain less histamine than mast cell granules (≈1 pg/cell compared to ≈3 pg/cell). In basophils, histamine is present in complex with chondroitin sulfate, which dissociates upon granule exocytosis (4). Histamine plays important roles in acute allergic reactions, but also in late-phase reactions, with physiological effects that include bronchoconstriction, mucus secretion and vasodilation.

1.5.2 Cytokines

The most well-known and important basophil-derived cytokines are the type 2 cytokines IL-4 and IL-13, which are generated mostly from *de novo* synthesis upon activation. Studies have shown that both IL-4 and IL-13 are important cytokines in protective immunity against several parasites. These cytokines also play critical roles in allergic diseases such as asthma and atopic dermatitis (3). The mechanism underlying both the protective and allergy-promoting effects of basophils is the type 2 skewing inflammatory effects of the cytokines (19). Basophil-derived IL-4 and IL-13 may also play roles in immunoglobulin class switching to IgE, further contributing to type 2 immunity (4). Furthermore, they can potently upregulate the expression of VCAM-1 on vascular endothelial cells, promoting leukocyte tissue infiltration (41). Several other basophil-derived cytokines are listed in Table 1.

1.5.3. Chemokines

In addition to cytokines, basophils can secrete multiple chemokines, thereby orchestrating inflammatory responses by recruiting other leukocytes to sites of inflammation. Basophil-derived chemokines include CXCL8, CCL5/RANTES and CCL3/MIP-1α, among others (4).

1.5.4 Phospholipid metabolites

In response to various stimuli, basophils can rapidly metabolize arachidonic acid to produce the proinflammatory lipid mediator LTC_4. Unlike histamine, LTC_4 is not preformed and stored in basophil granules. The rate at which LTC_4 is released however, is in the range of minutes after basophil activation, which is similar to the release of histamine. By this rapid release, LTC_4 is involved in the acute phase of inflammatory allergic responses (37). While basophils release less LTC_4 than histamine, LTC_4 is many times more potent than histamine in causing contraction of airway smooth muscle, making it an important and very potent basophil-derived mediator (37). In addition to LTC_4, the lipid mediator platelet activating factor (PAF) can be released by basophils, a response that is further enhanced by IL-3 (42). PAF is a mediator of anaphylaxis that can act on endothelial cells, resulting in vasodilation and increased vascular permeability, enabling further influx of immune cells into inflamed tissue (19).

1.5.5. Additional mediators

Basogranulin, a highly basic protein, is a unique component of basophil granules that is released alongside histamine upon degranulation. Basogranulin is recognized by the basophil-specific monoclonal antibody BB1 and can be used as basophil-specific marker (43). Despite associations with allergic disorders, the biological function of basogranulin remains elusive.

Granzyme B is a serine protease involved in granule-mediated cytotoxicity. IL-3 strongly promotes *de novo* synthesis of granzyme B in human basophils. Levels of granzyme B have been shown to correlate with IL-13 in bronchoalveolar lavage fluid in late-phase allergic responses in asthma patients after allergen challenge, suggesting a role in allergic inflammation, although details on the function and underlying mechanisms are lacking (44).

Basophils can promote the survival and activation of B cells by expressing B-cell activating factor (BAFF). IgD-mediated basophil activation strongly increases BAFF expression and immunoglobulin production (24). Through the release of BAFF, basophils in lymph nodes may contribute to B cell survival and autoantibody production in systemic lupus erythematosus, among others (45).

Among basophil mediators are several growth factors. Vascular endothelial growth factors (VEGFs) acts on VEGF-receptors expressed by endothelial cells. Basophils can secrete VEGF-A and VEGF-B, potentially contributing to angiogenesis and tissue remodeling in allergic inflammatory disorders and malignancies (4). The epidermal growth factor amphiregulin is secreted by basophils upon IL-3 stimulation. Amphiregulin can contribute to the tissue remodeling process in allergic inflammatory diseases such as asthma (46).

TABLE 1. Basophil inflammatory mediators

Inflammatory mediators secreted by basophils		
Mediator	Granule content or de novo synthesis	General effects
Cytokines		
IL-3	De novo synthesis	Autocrine basophil priming (synergistic functions in hematopoiesis)
IL-4	De novo synthesis and granule content	Promotion of Th2 differentiation, B cell activation, IgE isotype switch
IL-6	De novo synthesis	B cell promotion, promotion acute phase protein synthesis, Th17 differentiation
IL-13	De novo synthesis	Promotion of IgE synthesis, mucus production, fibrotic response
IL-31	De novo synthesis	Pro-inflammatory, puritus (itch), barrier dysfunction
Chemokines		
CCL5/RANTES	De novo synthesis	Leukocyte chemotaxis and tissue infiltration
CXCL8	De novo synthesis	
CCL3/MIP-1α	De novo synthesis	
Phospholipid metabolites		
Leukotriene C_4	De novo synthesis	Bronchoconstriction, increased vascular permeability, mucus production
Platelet Activating Factor	De novo synthesis	Increased vascular permeability, platelet activation
Others		
Histamine	Stored in granules	Bronchoconstriction, increased vascular permeability
Basogranulin	Stored in granules	Function unclear
Granzyme B	De novo synthesis	Granule-mediated cytotoxicity, degradation of endothelial cell-cell contacts allowing extravasation to sites of inflammation
BAFF	De novo synthesis	B cell survival and activation
VEGF-A / B	Stored in granules	Promotion of angiogenesis
Amphiregulin	De novo synthesis	Epidermal growth factor, tissue remodeling

Chapter 2 – Basophil function and dysfunction in disease

Over the past decades, basophils have been established as contributors to different disorders, most notably in allergic inflammatory disorders, constituting the largest area of basophil research. However, one of the first roles discovered for basophils was in immunity against helminth infections. More recently, basophils have been implicated in malignancies and autoimmune disorders. This chapter will describe a set of diseases in which basophils play (potential) roles, either protective or pathologic.

2.1 Protection against parasites

Helminths and ticks are multicellular parasites that can infect and cause large damage in the skin and intestinal mucosa. This is combatted by type 2 immune responses, providing protective host immunity against different endo- and ectoparasites. A key protective role for basophils has been established using basophil-depleting antibodies and basophil-deficient mouse strains.

2.1.1 Gastrointestinal helminths

Primary infections with gastrointestinal helminths are mainly resolved by IL-4- and IL-13-producing innate lymphoid cells type 2 (ILC2), whereas the response to a secondary infection with some helminths is dependent on IgE-induced release of IL-4 and IL-13 from basophils (47). Basophils can be activated by antigens binding to surface-bound antigen-specific IgE, by parasite-derived substances directly or by host-derived cytokines. The resulting secretion of IL-4 and IL-13 contributes to tissue repair, goblet cell hyperplasia and the formation of granulomas to encapsulate helminths, all contributing to effective parasite expulsion (3).

2.1.1 Ticks

Ticks are blood-feeding ectoparasites that can inject pathogens into the host upon feeding, thereby transmitting infectious diseases such as Lyme to humans. In some animal hosts, tick infestation is followed by the development of resistance, characterized by smaller and fewer ticks. Secondary infestation is accompanied by marked basophil infiltration in the skin, presumably linked to basophils being essential for development of acquired resistance (47). The exact mechanisms responsible for this specific protective role of basophils remain to be determined.

2.2 Malignancies

The notion of basophils being actively involved in tumor responses is relatively novel. Basophils have been found in human ovarian, gastric, lung and pancreatic cancers (48). There are studies showing protective roles of basophils, but also reports of basophils correlating with disease progression and negative outcomes. Which way the scale tips may be highly context dependent and requires further research.

2.2.2 Protective effects

In some cancers, basophils may have protective roles and correlate with positive outcomes. One study using a melanoma mouse model found that basophils promoted $CD8^+$ lymphocyte infiltration into the tumor microenvironment via the production of the chemoattractants CCL3 and CCL4 (49). This suggests that basophils promote tumor rejection in some settings. In human studies, higher numbers of circulating basophils and tumor-infiltrating activated basophils have been associated with better survival in patients with ovarian cancer (50).

2.2.2 Tumor promoting

Basophils may be involved in tumorigenesis by releasing proangiogenic factors, which promote the sprouting of new blood vessels. Activated human basophils can release considerable amounts of VEGF-A, VEGF-B and hepatocyte growth factor (HGF), all potent angiogenic factors (48). Another mechanism may be the promotion of alternatively

activated M2 macrophage differentiation in tumor-draining lymph nodes, which has been demonstrated for pancreatic ductal adenocarcinoma (51). Basophils can be recruited to tumor-draining lymph nodes and be activated to secrete IL-4. IL-4 is crucial for the polarization of Th2 cells and M2 macrophages, which can have tumor-promoting effects (51). Furthermore, there is some speculation on the contribution of extracellular DNA traps from basophils. Neutrophil extracellular traps are known to play tumor-promoting roles, but whether the same holds for basophil extracellular traps remains to be studied (48).

While their exact contribution is unclear, basophils are associated with different malignancies, the most well-characterized being chronic myeloid leukemia (CML), where basophils are associated with poor prognosis. In late stages of the disease, there may be basophilia and high levels of basophil-derived HGF and CCL3, both of which can promote the expansion of leukemia cells (52,53). Also in pancreatic cancer, higher numbers of infiltrating basophils are a marker of poor prognosis and associated with reduced survival (51).

2.3 Autoimmune disorders

Basophils are implicated in immune responses against autoantigens. Mostly, their pathogenic role appears to stem from the promotion of Th2 immune responses that enhance humoral responses and thus autoantibody production. The autoimmune disorder systemic lupus erythematosus (SLE) is characterized by multisystem inflammation leading to damage in the kidney, joints and skin. Autoreactive IgE and activated basophils are associated with disease activity and some patients have blood basopenia, possibly explained by basophil recruitment to secondary lymphoid tissues (45). Animal models indicate that basophils are essential for the development of lupus nephritis. In lymph nodes, activated basophils secrete IL-4, IL-13 and BAFF, enhancing type 2 responses and autoantibody production (45). This facilitates a feedback loop wherein autoreactive IgE-complexes activate circulating basophils, which infiltrate into secondary lymphoid organs through upregulated CXCR4 and adhesion molecule CD62L. In lymph nodes, these activated basophils secrete type 2 cytokines and B cell-promoting factors, further promoting autoantibody production from B cells (54).

Basophils may be involved in the pathogenesis of bullous pemphigoid, an autoimmune skin disorder with subepidermal blister formation. The number of basophils in skin lesions is increased in patients, and correlates with itch severity (55,56). Further research into the exact role of basophils in itch and skin autoinflammation may be an area of future research.

2.4 Allergic disorders

The most widely studied and acknowledged role for basophils is as an effector and regulator in allergic inflammatory disorders. Basophils have been implicated in IgE-dependent and IgE-independent allergic disorders. Here, different allergies with basophil-associations are described. Chapter 3 will describe more detailed the underlying mechanisms and functions that basophils have in allergic responses.

2.1.1. Inflammatory skin disorders

A large part of our knowledge on basophils in inflammatory skin disorders comes from mouse models. A study performed by Mukai et al. in 2005 demonstrated that basophils are essential in late phase allergic skin reactions (57). The group used a model of IgE-dependent chronic allergic inflammation (IgE-CAI) in which a single subcutaneous injection of antigen led to an immediate-, late phase- and delayed-onset ear swelling in passively sensitized mice. While mast cells were essential for the first two, basophils were crucial for the onset of the delayed reaction. The delayed ear swelling was not dependent on mast cells or T cells, indicating a primary role for skin-infiltrating basophils in IgE-CAI. In humans, basophils are recruited to skin lesions in a number of inflammatory skin disorders, including as atopic dermatitis (AD), urticaria and bullous pemphigoid (56). Especially in chronic urticaria, basophils can be highly present in skin lesions, and consequently there may be blood basopenia. Basophils can be used for classifying urticaria patients into responders or non-responders based on the degree of histamine release from basophils (58).

2.1.2. Inflammatory airway disorders

Allergic asthma is characterized by airway hyperresponsiveness, fibrosis and excess mucus production in the airways. While there are mouse models indicating that basophils are dispensable for the development of type 2 allergic airway hyperresponsiveness (59), some human studies have reported basophil accumulation in sputum and bronchial biopsies from patients with atopic asthma and fatal asthma (60,61). One study found that patients experiencing both early- and late-phase airway hyperresponsiveness had higher levels of allergen-induced basophils than patients developing only an early-phase response. Sputum basophils correlated with airway hyperresponsiveness in these patients (60). Basophil involvement in late-phase airway hyperresponsiveness marks basophils as potential contributors to asthma pathology.

2.1.3. Eosinophilic esophagitis

Eosinophilic esophagitis (EoE) is a chronic allergic disorder characterized by esophageal dysfunction, eosinophilia and a type 2 inflammatory response in the esophagus. There is evidence suggesting EoE is not IgE-mediated but instead depends on TSLP. As described earlier, TSLP is an important growth factor for the development of basophils. In EoE patients, TSLP levels are increased, and polymorphisms in the gene encoding TSLP have been reported (62). Accordingly, basophil counts in esophageal biopsies from patients with active EoE are higher than in controls, and both TSLP and basophils are essential for EoE-like disease in mice, suggesting an essential role for the TSLP-basophil axis in EoE pathology (63).

2.1.4. Food allergy

Food allergy patients can have symptoms ranging from gastrointestinal discomfort, itching and hives to acute anaphylaxis when exposed to food allergens. Food allergy is thought to be mediated largely by antigen-mediated crosslinking of allergen-specific IgE on mast cells. However, basophils are likely contributors to pathogenesis as well. In patients with peanut allergy treated with omalizumab, therapy response correlated with decreased basophil activity rather than mast cell activity (64). Mouse models of food-induced anaphylaxis indicate that both mast cells and basophils are important, as selective ablation of either one was able to improve symptoms (65).

Similarly to EoE, induction of food allergies may involve the TSLP-basophil axis. In a model of AD-like disease, epicutaneous sensitization to food allergens was associated with increases in TSLP-elicited basophils and the promotion of Th2 cytokine responses (66). By blocking TSLP or by depleting basophils, food allergy responses could be limited, suggesting that TSLP and basophils were required for the induction of food allergy (66).

While intestinal absorption of dietary proteins into circulation can take up to hours, systemic allergic reactions in patients with food allergy can occur within minutes. Local uptake of allergens in the oral cavity may explain this rapid onset of allergic responses. A study found that chewing peanuts, without swallowing, was sufficient for peanut allergens to be detected in serum after 10 minutes. Collected serum was able to induce considerable histamine release from human basophils *in vitro* (67). Basophils, as opposed to tissue resident mast cells, circulate in blood and could potentially be activated by rapidly absorbed allergens in the blood. This may better explain fast allergic reactions than food proteins being intestinally absorbed and subsequently activating intestinal mast cells.

Chapter 3 – Basophil immunomodulation in allergic disorders

3.1 Inducing allergic responses

3.1.1 Antigen presentation

In 2009, research interest in basophils experienced a surge after three independent back-to-back *Nature Immunology* publications demonstrated that murine basophils expressed MHC-II and could act as professional antigen-presenting cells to induce $CD4^+$ Th2 cells and thereby generate type 2 responses against helminths and allergens (Figure 2) (68–70). This novel role for basophils was however quickly disputed by other groups demonstrating that antigen-presentation by basophils was not essential for the early induction of Th2 responses. This was shown both in mice and in humans (71–73). Basophils did not take up or present antigen, and basophil depletion in mice only partially impaired Th2 responses, whereas dendritic cells (DCs) were required instead (71). It was found that human blood basophils lack features of professional antigen-presenting cells: there are no convincing signs of expression of MHC-II or costimulatory molecules (72). Some argued that the basophil cultures in the three *Nature* papers were likely contaminated with dendritic cells. In these studies, basophils were depleted based on FcεRI-expression, but it had since been shown that activated tissue DCs can also express high levels of FcεRI (74). Indeed, it was shown that FcεRI-positive DCs entered draining lymph nodes after allergen challenge in mice and that an anti-FcεRI antibody could effectively deplete these inflammatory DCs (71).

The later studies favor a more nuanced model in which DCs are the primary inducers of Th2 cells and basophils are accessory cells, contributing to Th2 differentiation, but not inducing this response through antigen-presentation (71). Basophils and professional antigen-presenting cells may cooperate, with basophils providing the Th2-promoting cytokine IL-4, and DCs presenting antigen to naïve T cells (Figure 2B). This IL-4-mediated contribution is relatively well-established, whereas controversies remain about the antigen-presenting capacities. Whether basophils can present antigen to naïve T cells may depend on experimental context and methods for cell isolation and depletion. Furthermore, antigen-presentation may be limited to murine basophils.

In 2017, Miyake et al. published results that were able to reconcile some of the discrepancies of basophil antigen-presentation. They proposed that (murine) basophils are indeed capable of antigen-presentation, not inherently, but through acquisition of this skill from DCs. MHC-II-antigen complexes and costimulatory molecules can be transferred from DCs to basophils in a cell contact-dependent process called trogocytosis (75). Basophils can then present the acquired complex, along with CD86, to naïve T cells, inducing Th2 differentiation (Figure 2C). This mechanism provides an explanation for antigen-presentation by basophils despite the limited gene expression of MHC-II and costimulatory molecules. However, whether this process of trogocytosis also occurs in humans remains to be studied, as well as its relative contribution compared to antigen-presentation by professional antigen-presenting cells.

3.1.2 Type 2 polarization

As described above, the role of basophils in the initiation of allergic responses may be more accessory than essential. Primarily through secretion of IL-4 and IL-13, basophils are potent promotors of type 2 allergic responses. Especially IL-4 is a central cytokine in the differentiation of naïve $CD4^+$ T cells into Th2 cells. IL-4 is produced by different cell types, including basophils, Th2 cells and type 2 innate lymphoid cells (ILC2). Basophils are candidates for providing the initial IL-4 required for inducing Th2 responses in lymph nodes (76). Indeed, murine and human basophils can secrete more IL-4 than Th2 cells on a per-cell basis, thereby affecting different processes and cells, including T cells, ILC2 and B cells, among others (77).

Most importantly, basophils provide IL-4 to naïve $CD4^+$ T cells, promoting their differentiation into Th2 cells (figure 2A). When basophils and naïve $CD4^+$ T cells are cocultured in the presence of dendritic cells, Th2 responses are strongly promoted, whereas coculture with IL-4-deficient basophils fails to elicit Th2 responses (78). In a mouse study with the protease allergen papain, basophils were rapidly recruited to draining lymph nodes prior to Th2

differentiation, where they secreted IL-4 (79). Basophil depletion impaired Th2 differentiation in lymph nodes, indicating the importance of basophils in inducing protease-allergen-induced type 2 responses *in vivo* (79). However, others have shown that basophils play only minor roles in primary Th2 responses to allergens, making this an unsettled issue (80).

Basophils and ILC2s may have overlapping or cooperative functions in the early phases of type 2 immunity. These innate cells can produce type 2 cytokines, affecting different leukocytes and tissue cells. However, there is evidence that basophil-derived IL-4 can promote ILC2 proliferation in inflammatory disorders. In a mouse model of atopic dermatitis, basophil responses preceded ILC2 responses and basophils were required for ILC2 proliferation and accumulation in the skin (81).

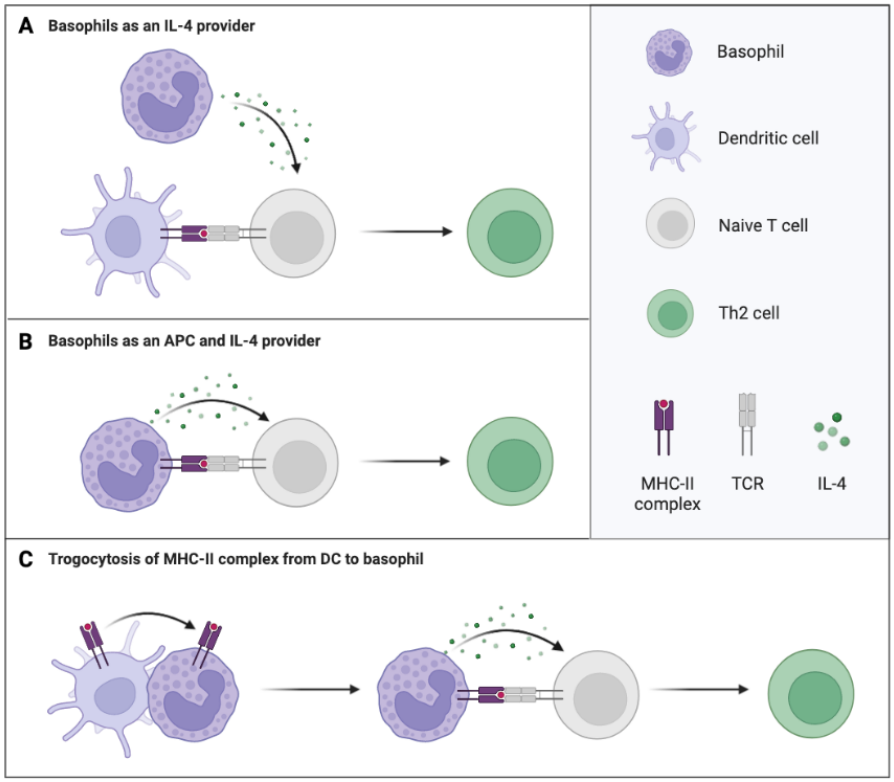

FIGURE 2. Basophils in Th2 differentiation.
(A) Basophils provide initial IL-4 to naïve CD4⁺ T cells to promote Th2 differentiation.
(B) In addition to providing IL-4, basophils may act as antigen-presenting cells under certain conditions by presenting antigen-MHC-II complexes to naïve CD4⁺ T cells.
(C) Basophils may acquire antigen-MHC-II complexes and costimulatory molecules from dendritic cells, after which they can present this to naïve T cells while providing IL-4 to promote differentiation of Th2 cells. IL-4, interleukin-4; APC, antigen presenting cell; MHC-II, major histocompatibility complex class II; DC, dendritic cell; Th2 cell, T helper 2 cell; TCR, T cell receptor. Figure made with BioRender.

3.2 Acute allergic responses

An IgE-mediated allergic reaction can occur in two phases. The acute, or early-phase response occurs within minutes after allergen exposure. The response can be systemic (anaphylaxis) or local (urticaria, wheezing, runny nose) and is largely mediated by degranulation of mast cells and to a smaller extent basophils. Mediator release can result in increased vascular permeability, vasodilation, smooth muscle contractions and mucus secretion, among others. When an allergen cross-links antigen-specific IgE on the surface of basophils, histamine, LTC_4 and PAF are rapidly secreted (Figure 3A). This rapid release implicates basophils as potential key players in acute allergic responses and anaphylaxis.

In mice, basophils appear to be important for IgG-mediated systemic anaphylaxis, whereas mast cells are required for classical IgE-mediated anaphylaxis. IgG-allergen immune complexes bind to basophils and induce release of PAF, which is the major mediator of IgG-mediated anaphylaxis (82). In human anaphylaxis, the number of circulating basophils is considerably reduced and inversely correlates with high levels of the basophil-chemoattractant CCL2 (83). The exact contribution of basophils to human anaphylaxis remains unclear however.

3.3 Late-phase allergic responses

The late-phase response following the acute allergic response generally occurs 6 to 12 hours later. The clinical symptoms may be similar to the initial response, characterized by selective immune cell accumulation at the site of allergen challenge and persistent release of inflammatory mediators. While mast cells are the key players in the early-phase by releasing histamine and prostaglandins, basophils contribute more to the late-phase response, which is characterized by high histamine and low prostaglandin levels. Marked basophil-infiltrations have been observed *in vivo* in late-phase responses to antigens in lungs, nose and especially skin (84). Basophils, rather than lymphocytes or eosinophils, may be a major source of IL-4 and IL-13 in these late-phase responses in tissues (37). Through the continued promotion of a Th2-skewing microenvironment, basophils contribute to the propagation of type 2 allergic responses. In late-phase allergic responses, basophils may be more a regulator of inflammatory responses rather than a potent effector cell (Figure 3B).

3.3.1. Recruiting other immune cells

Late-phase responses are characterized by immune cell infiltration of affected tissues, a process that can be orchestrated by basophils. By secreting a broad range of cytokines and chemokines, basophils are involved in the recruitment of other inflammatory cells to tissues. Basophils can cooperate with fibroblasts to promote eosinophil recruitment *in vitro*. This process is mediated by basophil-derived RANTES and IL-4-mediated eotaxin-expression in fibroblasts (85). Furthermore, after IgE-crosslinking, basophil-derived IL-4 and IL-13 can upregulate expression of VCAM-1 expression on vascular endothelial cells (41). Consequently, eosinophils infiltrate tissues through VCAM-1/VLA-4-interactions.

3.3.2. Promoting humoral responses

Basophils can provide stimulatory and helper signals to B cells, promoting humoral responses. In some studies, basophil depletion considerably impairs humoral responses, while in others basophils are not required for efficient humoral immunity (80,86). One way in which basophils can indirectly promote B cell responses, is by inducing a B-helper phenotype in $CD4^+$ T cells. Basophil expression of the costimulatory molecule CD40L and the secretion of IL-4 and IL-6 are required for providing this T cell-mediated help to B cells (86). T cell-independent induction of IgE synthesis from B cells has also been reported for basophils, largely mediated by CD40/CD40L-interactions and basophil-derived IL-4 and IL-13 (87). As described before, BAFF secretion by basophils can also promote B cell proliferation and class switching (45).

3.3.3. Delayed-onset response

Basophils have an essential role in the delayed-onset response in IgE-mediated chronic allergic inflammation (IgE-CAI). In mouse models of IgE-CAI, basophils are necessary and sufficient for delayed-onset allergen-induced ear swelling (57). This delayed-onset response is characterized by significant eosinophil and neutrophil infiltration in the skin, likely a result of basophil-induced chemotaxis. Mast cells are indispensable for immediate- and late-phase ear-swelling, but not involved in the third, delayed-onset reaction, indicating basophils and mast cells can have distinct functions in subsequent stages of allergic inflammation (57). In the same IgE-CAI model, an important role has been found for the murine basophil protease mouse mast cell protease-11 (Figure 3B) (mMCP-11). mMCP-11-deficient mice had less severe chronic allergic inflammation, characterized by reduced ear swelling and immune cell infiltration in the skin (88). Conversely, injection of mMCP-11 into the skin promoted tissue infiltration of basophils, eosinophils and macrophages, presumably through proteolytic cleavage of serum proteins (88). In the delayed-response, basophils can also promote the differentiation of M2 macrophages from inflammatory monocytes. In allergic skin lesions, basophil-derived IL-4 promotes differentiation of inflammatory monocytes into M2 macrophages that can exert anti-inflammatory effects, leading to resolution of allergic inflammation in IgE-CAI (Figure 3B) (89).

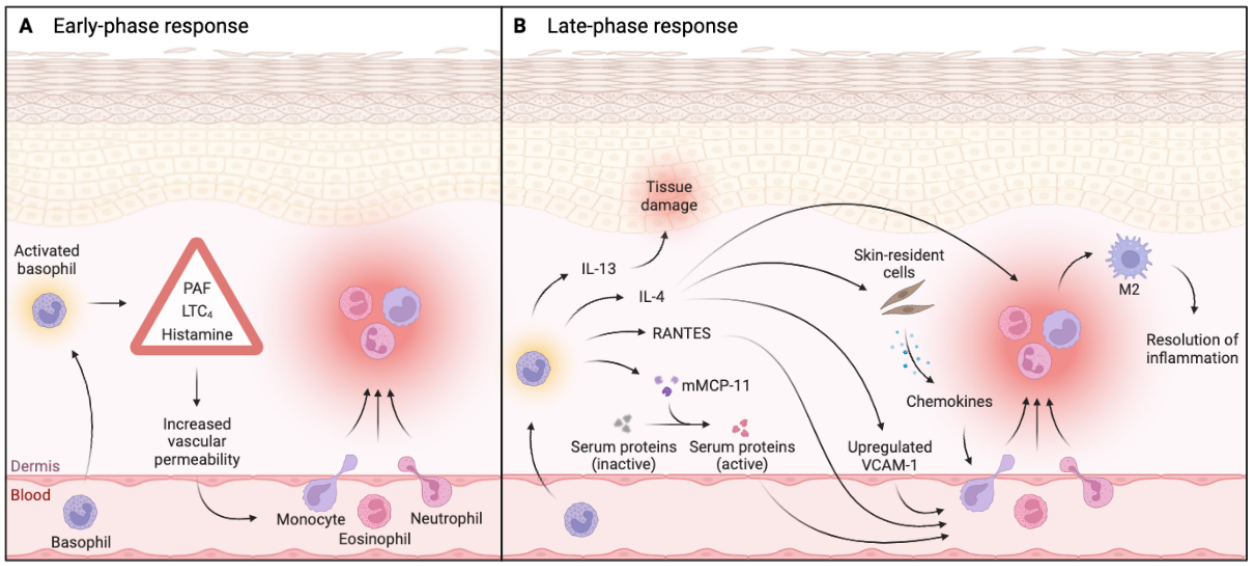

FIGURE 3. Basophils have effector and regulatory functions in different phases of allergic responses. **(A)** Basophils in the acute allergic response. In the early-phase response, basophils release vasoactive molecules increasing vascular permeability and recruiting other immune cells to the tissue of allergen challenge. **(B)** In the late-phase response, basophils accumulate in tissues, secreting cytokines and chemokines that can act on tissue-resident cells and other immune cells. A major function is recruitment of other leukocytes, promoting inflammation. In IgE-CAI, basophils recruit eosinophils and induce M2 differentiation from inflammatory monocytes. PAF, platelet-activating factor; LTC_4, leukotriene C_4, RANTES, regulated on activation normally T-cell expressed; mMCP-11, mast cell protease 11; VCAM-1, vascular cell adhesion molecule 1; M2, alternatively activated macrophage. Figure made with BioRender.

Chapter 4 – Basophils as a therapeutic target

Due to their diverse roles in different disorders ranging from induction of allergic and autoimmune disorders to anti-malignant effects, basophils present an interesting target for therapy. By targeting the different roles of basophils the disease progression or symptoms could be alleviated. However, there are currently no basophil-specific therapies, and the only way of targeting basophils is therefore through indirect targeting. Some current therapies for allergic disorders may be targeting basophil-associated pathways, indirectly affecting basophil function. The following sections describe several targeting strategies and their potential in treating inflammatory disorders.

4.1 Targeting IgE-signaling

Like mast cells, basophils can be (indirectly) targeted through FcεRI-pathways. The anti-IgE monoclonal antibody omalizumab, which is approved for the treatment of severe persistent asthma, nasal polyps and chronic idiopathic urticaria (CIU), primarily affects mast cells. By sequestering free IgE, omalizumab lowers total IgE levels, which leads to downregulation of FcεRI on mast cells and basophils and decreased release of inflammatory mediators. While omalizumab primarily targets mast cells, the effect of omalizumab on basophils may be responsible for early therapy effects observed in some allergic patients. One study showed that early clinical responses to omalizumab in adult food allergy patients may be due to suppression of basophils rather than mast cells (64). In other allergies, basophil FcεRI can already be downregulated several days after the first dose of omalizumab. Basophil FcεRI expression may be downregulated by almost 90% at seven days after treatment, whereas mast cell FcεRI downregulation requires several months (90). The explanation likely lies in the differences in life span of these cell types. Similarly, in CIU patients, the rapid effectiveness of omalizumab treatment can only be partially explained by effects on mast cells. Downregulation of basophil FcεRI and reversal of basopenia may account for some of the rapid clinical effects of

omalizumab in these patients (91). However, the exact role of mast cells versus basophils in CIU remains unclear, and thus also the exact mechanisms of action of omalizumab in the context of this disease.

Another method of disrupting IgE signaling is by engaging the inhibitory low-affinity receptor FcγRIIB (CD32b). Designed ankyrin repeat protein (DARPin)-based inhibitors make use this of strategy. These are engineered antibody mimetic proteins that can bind targets with high specificity and affinity, while overcoming some of the drawbacks of antibodies, such as large size. A bispecific DARPin targeting FcεRI and FcγRIIB, has been shown to inhibit *in vitro* IgE-mediated signaling and activation in human basophils as well as *in vivo* allergic responses in a mouse model of anaphylaxis (92). Engaging the inhibitory FcγRIIB receptor presents an interesting targeting strategy, although the *in vivo* feasibility of this approach has yet to be proven.

Alternatively, other points along the FcεRI signaling pathway could be targeted. An interesting candidate is Bruton's tyrosine kinase (BTK), an enzyme expressed by leukocytes that plays a role in FcεRI signaling in mast cells and basophils. Selective inhibition of BTK has been shown to decrease IgE-mediated *ex vivo* activation and mediator release of human basophils (93). In animal models, BTK-inhibition protects against IgE-mediated anaphylaxis, indicating potential relevance in the treatment of severe allergic disorders (94). In humans, short-term treatment with the BTK-inhibitor ibrutinib efficiently reduced skin-prick wheal areas and *ex vivo* IgE-mediated basophil activation in food-allergic adults (95). While major effects may be mainly due to mast cell targeting, basophils will likely be affected by BTK antagonists as well.

4.2 Targeting basophil mediators

Most current treatment strategies for allergic disorders are limited to inhibiting inflammatory mediators such as histamine and leukotrienes. Mast cells are the classical producers of these inflammatory mediators, although basophil-derived mediators may be (unintentionally) targeted as well. Targeting basophil-related molecules may prove beneficial in a number of allergic disorders, but as most of these molecules may also be produced by other immune cells, this approach does not truly target the basophils themselves.

Histamine and leukotrienes are the central mediators released by mast cells and basophils upon degranulation. These can be targeted by antihistamines and leukotriene receptor antagonists or synthesis inhibitors (96). In some allergic disorders these treatment strategies have yielded positive effects, but as both mast cells and basophils can release a plethora of other inflammatory mediators, benefits are sometimes limited. In basophils, some antihistamines can inhibit histamine release as well as activation and cytokine secretion (97). There are four histamine receptors (H1R-H4R), with H1R being the major histamine receptor involved in allergy. H1 antihistamines are commonly used to treat a broad range of allergies (96). More than mast cells, basophils may be inhibited by H2R agonists, whereas targeting H3R does not appear to affect basophil function (96).

There are treatments directed at cytokines that may (partially) originate from basophils. These include IL-4, IL-13 and TSLP. Dupilumab is a monoclonal antibody targeting the IL-4 receptor alpha chain, thereby blocking the signaling of the Th2 cytokines IL-4 and IL-13. Dual blockade of these two cytokines has proven effective in multiple allergic disorders, including asthma (98). While basophils may not be the major producers of these cytokines in allergic lesions, they are present and can secrete these cytokines, meaning that targeting these molecules could be indirect targeting of basophils.

TSLP presents a similar indirect basophil-derived target. While mucosal epithelial cells are the major source of TSLP in both inflammation and homeostasis, other cells, including basophils can contribute to TSLP production in allergic inflammation. Besides having Th2-promoting effects, TSLP can play a role in basophil activation, as described above. In EoE and food allergy models, targeting TSLP and TSLP-elicited basophils effectively diminishes symptoms, indicating they may be viable targets (63,66). The anti-TSLP monoclonal antibody Tezepelumab was very recently approved for the treatment of severe uncontrolled asthma. Ongoing (clinical) evaluation of TSLP-targeting in diverse conditions will increase our understanding of how TSLP, and potentially basophils, are involved in allergic disorders.

4.3 Targeting surface molecules

4.3.1. CD123 (IL-3Ra)

A more direct method of targeting basophils is with anti-CD123 (IL-3Ra) antibodies. The monoclonal antibody CSL362, originally developed against acute myeloid leukemia, binds to CD123 on basophils and plasmacytoid dendritic cells, leading to depletion of both cell types and inhibited IL-3 signaling (99). Basophils are critically dependent on IL-3/IL-3R signaling, and by preventing IL-3 from binding to CD123, basophil proliferation and activation may be targeted. In trials with CD123 targeting for acute myeloid leukemia, decreases in peripheral basophils have been reported (100). This approach also has potential in SLE, where targeting basophils may prove beneficial by reducing BAFF secretion and subsequent autoantibody production (99).

4.3.2. Siglecs

Different members of the Sialic-acid-binding, Ig-like lectin (Siglec) family are expressed by basophils. Of these surface proteins, we know most about Siglec-8, which is highly expressed by mast cells and eosinophils, and to a lesser extent also basophils (101). Engagement of Siglec-8 causes eosinophil apoptosis and inhibits mast cell mediator release (101). This inhibitory surface molecule has been proposed as a candidate for treatment of allergic disorders, by simultaneous targeting of mast cells, eosinophils and basophils. In mice, targeting of Siglec-8 or Siglec-F (the murine counterpart of Siglec-8) suppresses multiple aspects of acute and chronic inflammation (101). Whether and how targeting of Siglec-8 affects basophils remains to be studied. In addition to Siglec-8, basophils express Siglec-7, although engagement of this receptor only causes moderate inhibition of basophil activation (102). As with the strategies highlighted above, basophils are unlikely to be the major target of anti-Siglec therapies. It could however still be that the effect on basophils contributes to overall anti-inflammatory effects of these therapies.

4.3.3. CRTH2

The prostaglandin D2 (PGD_2) receptor CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells) is expressed on basophils and other leukocytes. PGD_2 is secreted by mast cells upon IgE-mediated activation. CRTH2 is another target that is not basophil-specific but can diminish some basophil-associated effects such as high IL-4 and IL-13, as well as basophil degranulation and chemotaxis (103). Several CRTH2 antagonists have been developed and studied in allergic disorders, some of them with promising effects in asthma and allergic rhinitis (103).

Gaining a better understanding of the diverse effector functions and regulatory roles basophils play in (allergic) disease will help in uncovering targeting strategies directed at basophils specifically, instead of having to rely on downstream effector molecules. The contribution of basophils to pathology needs to be established firmly, giving a better idea of whether targeting basophils is likely to contribute to therapeutic effects and altering disease progression. If basophils prove to be redundant in some disorders, targeting would be unlikely to contribute to effective treatment.

Chapter 5 – Challenges and future perspectives in basophil research

5.1 The question of contribution

Basophils have long been overshadowed by mast cells but we now know that they are distinct cells with some separate functions. Our understanding of basophil biology and properties has increased considerably over the past two decades, but plenty of questions remain. They are clearly associated with a broad range of disorders, from allergies to hematological malignancies, but better characterization of the exact roles played by basophils is needed to better determine the diagnostic and therapeutic value of these cells.

Basophil research appears to have experienced a peak around 2009 and successive years, with interest surging around the three *Nature Immunology* papers demonstrating antigen-presenting capacities of basophils. In the years after that, many reviews and articles announced basophils were back, with increased recognition of the basophil in different human diseases. In recent years however, research on basophils as classic allergic effector cells has decreased, whereas there is now more focus on their immunoregulatory potential. Furthermore, the role of basophils in very diverse diseases, such as cancer and autoimmune disorders, has been a topic of recent research. A recurrent question is whether basophils are truly essential for immune responses, or whether they are assesory cells.

As we have seen, basophils can have a broad range of both effector and regulatory functions in allergic responses (Figure 4). However, almost all these functions can also be performed by other cells, sometimes even better. Basophils are a major source of the type 2 cytokines IL-4 and IL-13, but other cells, such as ILC2 and Th2 cells, are prominent producers as well. Basophils may be essential initial providers of IL-4 required for the induction of type 2 responses. However, this role is not basophil-exclusive, as natural killer T (NKT) cells have also been postulated to be an important source of early IL-4 in allergic sensitization (36). With regards to histamine and lipid mediator release, basophils are overshadowed by mast cells, the classical histamine-releasing cell. Despite this, basophils and their mediators may be more important in late phase responses, whereas mast cells have essential roles in acute allergic responses. Furthermore, as blood-circulating cells, basophils are uniquely positioned to be major effector cells for blood antigens, for example in the case of venom allergies or drug hypersensitivity. Similarly, rapid uptake of food allergens in the blood and subsequent rapid basophil activation and degranulation could be involved in food allergic reactions (67). In such cases, the response of tissue mast cells does not provide adequate explanation of the rapidly occurring symptoms, whereas basophil responses can be hypothesized to be of greater importance.

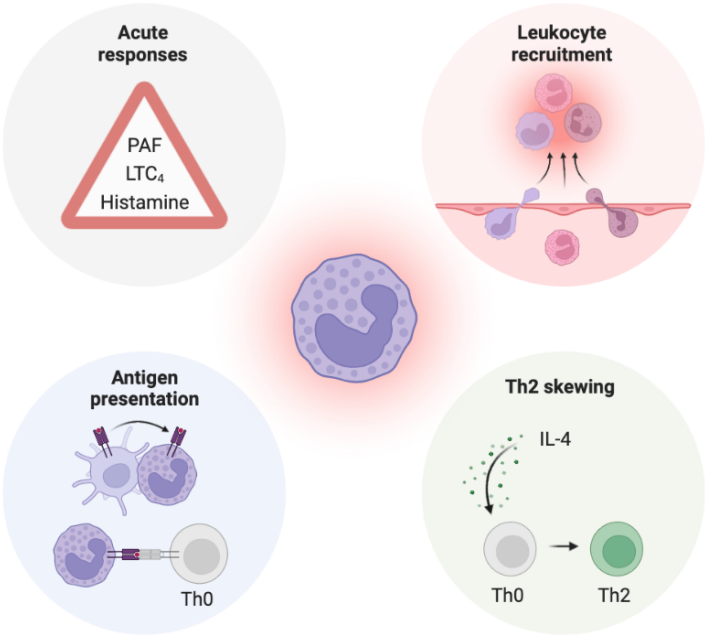

FIGURE 4. The versatile (potential) roles of basophils include effector functions in acute allergic responses, orchestration of leukocyte recruitment in late-phase responses, antigen presentation and promotion of Th2 differentiation. Further research is needed to elucidate the extent of basophil contribution to these processes, which may also be performed by other cell types than basophils. PAF, platelet-activating factor, LTC4, leukotriene C4; Th0, naïve T cell; Th2, T helper 2 cell, IL-4 interleukin-4.

Lastly, while there is evidence of antigen-presenting capacities of murine basophils, this function does not seem to be a prominent function of these cells, especially not for human basophils. Professional antigen-presenting cells like dendritic cells are better equipped and essential for proper antigen-presentation. All this amounts to basophils having broad functional capabilities, but the question remains whether they actually do these things and how relevant their contribution is. This has been on the table for more than a decade, and was aptly highlighted by a *Nature Immunology* review more than 10 years ago already that was termed 'Basophils: what they 'can do' versus what they 'actually do'' (104). While our understanding of basophils has increased considerably, the question remains. On one hand there are studies reporting that basophils are essential in early Th2 responses and protection against certain parasites (68,69) but on the other hand are reports of basophils having only minor roles in Th2 responses and instead being essential for chronic allergic inflammation (71,80). Different models yield different results and many of these discrepancies have not yet been solved convincingly. Translation to different human disease endotypes is an additional challenge. Human (allergic) disorders are multifaceted and can have different endotypes, e.g. eosinophilic and non-eosinophilic asthma. We can hypothesize that basophils are especially involved in some disease subtypes and less in others. The existence of such a basophilic endotype in allergic disease would be interesting in light of basophil-targeting therapies.

A large part of the recent literature on basophils deals with their value as a diagnostic or prognostic tool. The *ex vivo* basophil activation test (BAT) uses flow cytometry to measure the degree of degranulation upon stimulation with different allergens and can be used to characterize the allergic status of a patient (105). BAT is a highly accurate and reproducible tool for diagnosing food allergy and monitoring immunomodulatory therapies and provides several advantages over classical patient allergen challenges (105). This marks basophils as highly useful cells, even if their exact contributions to allergic responses remain unclear.

So far, there appears to be no evidence of humans that are completely basophil-deficient. Blood basophil counts may be low in patients with an (acute) allergic reaction, which is likely caused by basophils being recruited from the blood into inflamed tissues and peripheral lymph nodes. Total absence of basophils however, has not been reported, suggesting an evolutionary relevance and non-redundancy.

5.2 The challenge of translational research

Basophil research has long been hampered by the lack of methods allowing for efficient and specific basophil depletion. There are no natural mouse mutants that have basophil deficiencies and as a result, depleting antibodies are often used to study the role of basophils in different conditions. Such antibodies include the antibody MAR-1, targeting FcεRI, and the antibody Ba103, targeting the activating receptor CD200R3 (3). A major limitation of this approach is that these antigens are not exclusively expressed by basophils, meaning that other cell types including mast cells and dendritic cells can also be depleted. As described above, this problem of non-selective basophil depletion may have played a role in the controversy around basophils as antigen-presenting cells (74). Related to the challenges of selective depletion, the selective targeting of basophils has proven difficult so far, as was described in the previous chapter. This may be associated with most of the functions of basophils also being carried out by other cell types. There are basophil-specific (activation) markers, such as basogranulin, CD123 and CD63, but specific therapeutic targeting of basophils has been difficult so far. Herein lie some big challenges, but also opportunities, for future research.

An additional problem that needs to be accounted for is the fact that murine and human basophils are not the same. Differences have been found in the activation and responses of murine and human basophils. Differences in surface molecule expression, cytokine and mediator production and effector functions (e.g. antigen presentation) have been reported (48). Some functions have only been demonstrated for murine and not for human basophils. This can make translation to humans challenging. There are examples of valuable translational experiments, one of them being a 2010 *Nature Medicine* study of basophils in SLE development (45). In this study, findings in a mouse model of lupus-like nephritis are backed by data showing that the basophil characteristics and associations observed in the mouse model are also present in patients with SLE. Incorporation of a translational step is a valuable addition in a research field that is largely dominated by murine studies.

Chapter 6 – Closing remarks

While basophils were discovered almost 150 years ago, we have just recently started to appreciate the multifaceted roles of these cells in health and disease. The view has expanded from basophils as histamine-releasing effector cells in allergic reactions to cells with complex immunoregulatory functions, forming a link between innate and adaptive immunity. A large part of immunomodulatory capacities of basophils are mediated by chemokines and IL-4, a key instructor of adaptive immune responses. It seems likely that the focus will shift further away from classical effector functions like histamine release, into immunoregulatory properties. These rare, yet versatile leukocytes are engaged in cellular crosstalk with T cells, B cells, dendritic cells, ILC2 and more. In the context of immunomodulation, the potential contribution of basophils to allergic and non-allergic disorders such as cancer and autoimmunity is an area of ongoing research. Currently, basophils are mostly used as a well-established diagnostic and monitoring tool for allergies. Further research is needed to fully characterize the extent of basophil contribution to human health and disease, telling us whether basophils present a viable target for treatment. Meanwhile, the search for basophil-specific targeting strategies continues, with ample room for new developments.

As to the fiction and the facts regarding the roles of basophils, we are still missing multiple facts when it comes to these enigmatic cells. Their broad functional capacities could mean that these cells provide a sort of back-up to keep the human immune system resilient, should a part of the system falter. Whether certain functions of basophils fall under fact or fiction, may strongly depend on the situation and conditions. Until further research elucidates the exact contribution and mechanisms of basophil involvement in different responses, we may view basophils as non-redundant immunomodulatory cells that contribute to a variety of immune responses, if not as an essential cell, then definitely as a resourceful contributor.

Plain language summary

Basophils are one of the several types of white blood cells in the immune system. These cells make up a minority of circulating immune cells and have therefore long been overshadowed by other cells. They were first identified by the researcher Paul Ehrlich in 1879, but it is only in the last few decades we have started to pay more attention to these rare immune cells.

These cells are so called granulocytes, which means that they contain granules that are filled with preformed inflammatory molecules. The most well-known of these inflammatory molecules is histamine. Histamine is a key player in allergic responses and can cause symptoms such as itching, sneezing or coughing. When a basophil is activated these granules are thrown out of the cell, which rapidly releases these preformed inflammatory molecules to the cells surroundings. This gives basophils the ability to be very fast responders in certain situations. Basophils can also produce and release other molecules from the point of their activation. These molecules have different functions, ranging from tissue damage to the recruitment of other immune cells.

The most well-known role for basophils is in allergies. An allergic reaction is caused by exposure to something that triggers a strong immune response. The molecule that causes the allergic response is known as an 'allergen'. The immune system responds to an allergen by setting in motion different immune processes, including basophil responses. Basophils migrate to inflamed tissues, are activated and perform various actions. Firstly, they can release inflammatory molecules such as histamine. As a result, blood vessels can become more permeable, allowing other immune cells to enter the site of inflammation. Secondly, basophils can actively recruit other immune cells by secreting molecules that attract immune cells. Another major function of basophils is in the early phase of certain immune responses, for example in allergies. Basophils provide some of the molecules that are necessary for starting an allergic response. If there would be no basophils, some immune responses would not be very efficient or would not even be started at all.

Because of their involvement in allergic disorders, basophils are potential targets for treatment. The trouble is that there is currently no good way of specifically targeting basophils and effectively preventing or limiting their activity in humans. There are potential strategies in development, but further research and testing is required to bring basophil-targeting therapies to the clinic to help treat patients with allergic disorders.

While basophils appear to be important for some immune responses, there is research showing the opposite. Conflicting data on the importance and the exact roles off basophils makes it difficult to draw clear conclusions with regards to these cells. There are many challenges to overcome before we have a complete understanding of these rare immune cells and their roles in health and disease.

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