

4. IgE, mast cells, basophils, and eosinophils

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IgE, mast cells, basophils, and eosinophils constitute essential elements in allergic inflammation. Allergen-specific IgE, synthesized in response to allergens in the environment and in susceptible individuals, becomes fixed to high-affinity receptors on cellular membranes, especially of mast cells and basophils. If these receptor-bound IgE molecules are aggregated on reexposure to specific allergen, these mast cells and basophils produce mediators that result in the allergic response. Principal among the cells drawn to sites of mediator release is the eosinophil. (J Allergy Clin Immunol 2003;111:S486-94.)

Key words: *IgE, mast cell, eosinophil, basophil, high-affinity IgE receptor, hematopoiesis, kit, immediate hypersensitivity, histamine, leukotrienes*

IGE

IgE (immunoglobulin E) reagenic antibody has an approximate molecular weight of 190 kd, shows no transplacental transfer, and, unlike other immunoglobulins, does not activate complement by the classic pathway. IgE is thermolabile and will not sensitize after it has been heated to 56°C for several hours. IgE is principally known for its ability to bind with high-affinity to its specific receptor (R) FcεRI, found in its complete form (αβγ₂) on the membranes of mast cells and basophils.¹

The concentration of IgE in serum is the lowest of the five human immunoglobulin isotypes (0-0.0001 g/L, constituting 0.004% of total serum immunoglobulin) and is highly age dependent. Serum IgE concentration is low in cord serum (<2 kIU/L, <4.8 mg/L) and rises with age until a person is 10 to 15 years old. Those with an allergic predisposition show an earlier and steeper rise. Total serum IgE levels decline from the second to the eighth decades of life. Approximately 50% of total body IgE is estimated to exist in the intravascular compartment. IgE has a half-life of 1 to 5 days in peripheral blood.^{1,2}

IgE synthesis

B cells initially produce IgM antibodies but after appropriate stimuli alter the isotype of the antibody produced while retaining antigenic specificity through sharing of the same variable (VDJ) region. This “isotype switching” is efficient, in that it allows a single B-cell

Abbreviations used

CD40L:	CD40 ligand
EDN:	Eosinophil-derived neurotoxin
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
IFN:	Interferon
Ig:	Immunoglobulin
IL:	Interleukin
ITAM:	Immunoreceptor tyrosine-based activation motif
LT:	Leukotriene
MBP:	Major basic protein
MC _T :	Mast cell (tryptase containing)
MC _{TC} :	Mast cell (tryptase and chymase containing)
PGD ₂ :	Prostaglandin D ₂
R:	Receptor
SCF:	Stem cell factor
STAT-6:	Signal transducer and activator of transcription 6
TNF:	Tumor necrosis factor
VCAM:	Vascular cell adhesion molecule
VLA:	Very late antigen

clone to produce antibodies of the same specificity but distinct effector functions residing in the constant part of the heavy chain. This process consists of splicing and rejoining of genomic DNA to juxtapose VDJ elements to C-region exons that encode, in the case of IgE, the ε chain determining the IgE isotype.

For IgE synthesis, two signal types are required. Signal 1 is provided by the cytokines interleukin (IL)-4 and IL-13, which activate transcription at a specific immunoglobulin locus. The second signal is provided by ligation of CD40 on B cells, which in turn activates DNA switch recombination. Both signals are presented to B cells by T cells.^{3,4}

The process is initiated when an allergen binds to allergen-specific IgM on a B cell, which then processes the allergen. When the B cell next presents fragments of this allergen in the context of MHC class II molecules to the T-cell receptor-CD3 complex on a T_H2 cell, the T cell rapidly expresses IL-4 and CD40 ligand (CD40L, CD154). CD40L engages CD40 expressed on the B cell. This interaction results in expression of B7 on B cells, which binds to CD28 on the T cell, resulting in upregulation of T cell-derived IL-4 (Fig 1). IL-4, in turn, binds to the IL-4 receptor (IL-4R) on the B cell, triggering ε-germline transcription. The CD40-CD40L interaction activates DNA recombination at the targeted ε-switch region, which is followed by secretion of allergen-specific IgE.³⁻⁵

IL-4 activation of ε-germline transcription is initiated when it binds to B cell IL-4R, a heterodimer of an α and a γ chains, where the γ chain is common to IL-2, IL-7, IL-9, and IL-15. Dimerization of IL-4R by IL-4 leads to

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activation of Janus kinase 1, attached to the α chain, and Janus kinase 3, attached to the γ chain. The α chain is then phosphorylated to generate docking sites for signal transducer and activator of transcription 6 (STAT-6). Tyrosine phosphorylation induces STAT-6 homodimerization, which then results in translocation of STAT-6 to the nucleus, where it binds to IL-4-responsive promoter elements and activates transcription.⁵ Germline transcription and IgE class switching are markedly impaired in STAT-6-deficient mice.⁴ In addition to STAT-6, nuclear factor κ B and B-cell-specific activator protein elements must be present and functional in the ϵ -promoter, where nuclear factor κ B activation follows ligation of CD40. BCL-6, a POZ/zinc-finger transcription factor, can repress ϵ -germline transcription. BCL-6-deficient mice have increased IgE isotype switching.⁴ Polymorphisms in the gene for BCL-6 have been associated with atopy.⁶

In addition to IL-4, IL-13 induces ϵ -germline gene transcription in human B cells.⁷ In human B cells, IL-13 signals through the type II IL-4 receptor, which consists of the IL-4R α chain and a unique ligand-binding IL-13R α 1-chain.

A critical role for CD40-CD40L interactions in IgE synthesis and in isotype switching in general is shown by patients with the X-linked hyper-IgM syndrome.^{3,4} These patients are deficient in CD40L. As a consequence, their B cells are unable to produce IgA, IgG, or IgE.

Human mast cells and basophils have been variously reported to secrete IL-4, IL-13, or both, and to express some CD40L. These observations thus suggest that these cells may interact with B cells to provide signals for IgE synthesis or amplification. Because basophil and mast cell IL-4 or IL-13 production depends on the aggregation of Fc ϵ RI through antigen-specific IgE, this mechanism is more attractive for amplification of IgE synthesis. This mechanism would not appear to be allergen specific, but rather to induce a polyclonal response.³ This hypothesis is consistent with the observation that the IgE response in hyper-IgE states is polyclonal.

IgE receptors

There are two distinct receptors for IgE, the low-affinity IgE receptor (Fc ϵ RI; CD23) present on B cells and the high-affinity IgE receptor (Fc ϵ RI). Fc ϵ RI on mast cells and basophils is a tetramer ($\alpha\beta\gamma_2$), whereas it is expressed in a trimeric form ($\alpha\gamma_2$) on antigen-presenting cells, such as monocytes, Langerhans cells, and peripheral blood dendritic cells. The density of human basophil Fc ϵ RI α expression correlates with serum levels of IgE,⁸ because binding of IgE to Fc ϵ RI stabilizes the receptor at the cell surface. Fc ϵ RI-IgE interactions may also promote mast cell survival.

The Fc fragment of IgE binds to the α chain of Fc ϵ RI. The single Fc ϵ RI β chain, which has two immunoreceptor tyrosine-based activation motifs (ITAMs), amplifies the signaling of this receptor and is associated with lyn. Two disulfide-linked γ chains each contain two ITAMs, which are phosphorylated after aggregation of receptor-bound IgE. Syk kinase then binds to the γ chain ITAM, leading to its activation.⁹ Protein levels of syk are unde-

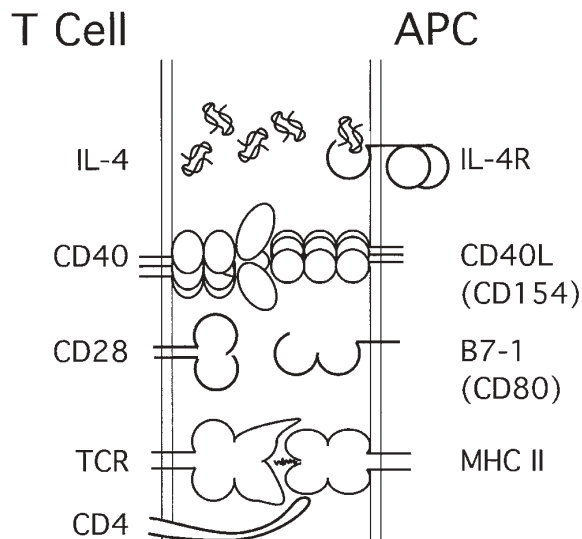


FIG 1. Molecular interactions between T_H2 cells and B cells required for IgE synthesis. APC, Antigen-presenting cell; TCR, T-cell receptor.

tectable in “nonreleaser” basophils that do not degranulate in response to Fc ϵ RI cross-linking.¹⁰ Nonreleaser basophils are rescued by IL-3, which causes syk to be expressed. Lyn bound to the β ITAM is also phosphorylated after Fc ϵ RI aggregation.

The lyn-syk-dependent signaling cascade involves phosphorylation of multiple substrates. These include adaptor molecules such as src homology collagen-like protein, vav, and linker for activation of T cells; phospholipases such as PLC γ_1 and PLC γ_2 ; tyrosine kinases such as focal adhesion kinase and Bruton tyrosine kinase; and protein or inositol phosphatases such as SHP1, SHP2, and SHIP. Coligation of inhibitory receptors, such as Fc γ RIIb with Fc ϵ RI, results in downregulation of secretory responses in human basophils.¹¹

Measurements of total and specific IgE

Total IgE levels are influenced by age, genetic predisposition, ethnicity, immune status, season of the year, and some disease processes (see also Chapter 23). Increased IgE levels are found in parasitic diseases, such as schistosomiasis and hookworm; infections, such as allergic bronchopulmonary aspergillosis and Epstein-Barr virus mononucleosis; cutaneous diseases, such as bullous pemphigoid; neoplastic diseases, such as Hodgkin disease and IgE myeloma; immunodeficiency diseases, such as Wiskott-Aldrich syndrome, hyperimmunoglobulinemia E syndrome, thymic hypoplasia (Di George syndrome), and cellular immunodeficiency with immunoglobulins (Nezelof syndrome); and several other diseases, such as nephrotic syndrome, cystic fibrosis, Kawasaki disease, and infantile polyarteritis nodosa.² Collectively such information dictates that measurement of total serum IgE level is of limited value as a screening test for allergic disease.

Total IgE is usually measured with a two-site non-competitive immunometric assay. Here a solid-phase

anti-IgE is used in molar excess to capture IgE from serum. A different antihuman IgE labeled with enzyme, fluorophor, or radionuclide is added in molar excess to detect bound IgE (see Chapter 23).

Antigen-specific IgE is usually measured by skin testing or through the use of allergen-specific IgE measurements in serum. Administration of allergen extract to skin uses either prick-puncture or intradermal injection. Prick-puncture skin testing involves placing a drop of allergen extract on the skin, followed by needle prick-puncture to introduce the allergen into the dermis. An immediate wheal and erythema reaction is read at 15 to 20 minutes. An intradermal skin test involves injection of 0.02 mL of diluted antigen intracutaneously through a syringe with a 26- to 27-gauge needle. The skin reaction is then read at 15 to 20 minutes (see Chapter 23).

A number of commercial assays are available to detect allergen-specific IgE antibody in serum. Such assays are particularly useful when skin testing cannot be used because of extensive skin disease, current medical therapy, significant dermatographism, or use of an extract believed to have a high probability of inducing a systemic reaction in the subject to be tested.

Allergen-specific IgE levels in a given individual depend on the degree and duration of exposure to both the allergen in question and to cross-reactive allergens. Allergen-specific IgE levels, as a rule, peak about 4 weeks into seasonal pollen exposure and gradually fall until the next pollen season. IgE levels also usually decline during immunotherapy. It should be remembered that many individuals have positive responses to tests for allergens but show no clinical reactivity to these allergens.

MAST CELLS

The mast cell is a tissue-based inflammatory cell of bone marrow origin that responds to danger signals of innate and acquired immunity with immediate and delayed release of inflammatory mediators.¹² Mast cells have long been implicated in the pathology and mortality of anaphylaxis and other allergic disorders by virtue of their ability to be activated through FcεRI-bound antigen-specific IgE and their concentration at surfaces that interface with the external environment.

The human mast cell is generally ovoid or irregularly elongated in tissue. The characteristic feature of the mast cell is the presence of dense cytoplasmic granules that occupy the cytoplasm. In human beings, these granules contain lattice or scroll-like structures. The nucleus of the mast cell is reminiscent of the plasma cell. Granules exhibit metachromasia when stained with toluidine blue, appearing reddish blue. Mast cells are relatively abundant in skin, thymus, lymphoid tissue, lung, nasal mucosa, conjunctiva, uterus, urinary bladder, tongue, synovia, and mesentery; around large and small blood vessels; and in the subserosal and submucosal layers of the digestive tract. Mast cells occur chiefly in the loose connective tissue surrounding blood vessels, nerves, and glandular ducts and under epithelial, serous, and synovial membranes. In general, mast cells

are scant in parenchymous tissues. In the lungs, mast cells are found both in bronchial airway connective tissues and in peripheral intra-alveolar spaces. In the skin, mast cells appear in greatest number near blood vessels, hair follicles, sebaceous glands, and sweat glands. Human skin contains approximately 10,000 mast cells per cubic millimeter.¹³

Mast cells in human tissues are divided into two major subtypes according to the secretory protease content, designated as MC_T or MC_{TC} according to the presence of tryptase with or without chymase. MC_{TC} cells thus contain tryptase and chymase, as well as carboxypeptidase and cathepsin G. MC_T cells contain only tryptase. Tryptase staining thus identifies all mast cells in tissues and has become the principal method of visualizing mast cells. MC_{TC} cells predominate in skin and small bowel submucosa. MC_T cells predominate in normal airway and small bowel mucosa. MC_T cells appear selectively attenuated in the small bowel of patients with end-stage immunodeficiency diseases.¹⁴ Human mast cells are characterized as Kit⁺ (positive for receptor for stem cell factor [SCF]) and FcεRI⁺. They express a variety of membrane receptors, depending on their tissue source, state of differentiation, and conditions of culture. Human resting mast cells express the high-affinity IgE receptor (FcεRI) and FcγRIIb (CD32). After exposure to interferon (IFN)-γ in vitro, they express the high-affinity receptor for IgG (FcγRI, CD64). Mast cells may also express C3a and C5a receptors. They may be similarly positively staining for cytokine receptors (IL-3R, IL-4R, IL-5R, IL-9R, IL-10R, granulocyte-macrophage colony-stimulating factor [GM-CSF]R, IFN-γR), chemokine receptors (CCR3, CCR5, CXCR₂, CXCR4),¹⁵ and nerve growth factor receptors, among others.

Development

Human mast cells develop from CD34⁺ pluripotent stem cells, which are Kit⁺. Mast-cell precursors circulate in the blood and lymphatics and home to tissues. There they survive and mature under the influence of SCF produced locally by stromal cells, including fibroblasts and endothelial cells. SCF occurs in both membrane-bound and soluble forms, both of which are active. Mast cells in tissues may have their survival, maturation, and biologic expression influenced by such cytokines as IL-4, IL-5, and IFN-γ. IL-5 promotes mast-cell proliferation in the presence of SCF, whereas IFN-γ exposure upregulates FcγRI on the mast cell membrane. IL-4 has been reported both to enhance mast-cell proliferation and to induce apoptosis, depending on the presence of other cytokines, such as IL-6.

Mast cells increase in number severalfold in association with IgE-dependent immediate hypersensitivity reactions, including rhinitis, urticaria, and asthma. Small increases in mast cell number have been noted in association with connective tissue disorders, such as rheumatoid arthritis and scleroderma, infectious diseases, such as tuberculosis and syphilis, neoplastic diseases, such as lymphoma and leukemias, and osteoporosis, chronic liver disease, and chronic renal disease.¹⁶ The most striking increase in mast cells (more than severalfold) occurs in association with mastocytosis.

Activation

Mast cells express a complete and functional FcεRI receptor ($\alpha\beta\gamma_2$), aggregation of which leads to mast-cell activation, granule exocytosis, and mediator release. Mast cells may also be activated by C3a and C5a through C3aR and C5aR (CD88),¹⁷ nerve growth factor through TRKA,¹⁸ and IgG through FcγRI.¹⁹ Activation through any of these receptors leads to histamine release, eicosanoid synthesis, and cytokine gene expression (Fig 2). The extent and pattern of mediators released depends on the signal, its intensity, and the cytokine milieu in which the activation takes place. For instance, mediator release and generation are enhanced in the presence of SCF.

Mediators

Mediators produced by human mast cells have classically been divided into three categories: preformed mediators, newly synthesized lipid mediators, and cytokines. These categories are not absolutely exclusive, with at least one cytokine, tumor necrosis factor (TNF)- α , occurring both preformed and as a newly synthesized molecule.

Preformed mediators are packaged within secretory granules. At activation, granule contents are released into the extracellular environment within minutes. Principal granule constituents include histamine, serine proteases, carboxypeptidase A, and proteoglycans (heparin and chondroitin sulfate E). Human mast cells contain approximately 2 to 5 pg of histamine per cell. Histamine in granules is found in ionic association with acidic residues of the glycosaminoglycan side chains of heparin and chondroitin sulfate E, and it dissociates from these glycosaminoglycans in extracellular fluids by exchanging with sodium ions.²⁰ Histamine has effects on smooth muscle (contraction), endothelial cells, nerve endings, and mucous secretion. It is rapidly degraded to N-methyl histamine, methylimidazole acetic acid, and imidazole acetic acid. Heparin and chondroitin sulfate proteoglycans are believed to aid in storage of preformed molecules, which dissociate from these proteoglycans at variable rates in physiologic buffer solution. Heparin itself is capable of anticoagulation through binding to antithrombin 3.

Most of the protein in mast cell granules is made up of four neutral proteases: tryptase, chymase, carboxypeptidase, and cathepsin G. Tryptase, a tetramer with a molecular weight of 116 to 130 kD with subunits of 29 to 36 kD (the heterogeneity is due primarily to differential glycosylation), is stabilized by its association with heparin and other proteoglycans. The function of tryptase *in vivo* is unknown, but *in vitro* it can cleave C3 and C3a, activate fibroblasts, and promote accumulation of inflammatory cells. Both α and β tryptases have been described, and it has been said that the α form is constitutively secreted, whereas the β form is released during degranulation. The accuracy of this conclusion remains to be conclusively demonstrated.

The major lipid mediators synthesized by mast cells include prostaglandin D₂ (PGD₂), the major cyclooxygenase product, and the lipoxygenase product leukotriene (LT)

C₄. Extracellular peptidolytic processing of LTC₄ yields the active metabolites LTD₄ and LTE₄. Skin mast cells produce more PGD₂ than LTC₄, whereas the opposite is true of mast cells from the lung. PGD₂ and LTC₄, LTD₄, and LTE₄ are all bronchoconstrictors. LTC₄, LTD₄, and LTE₄ also enhance vascular permeability.⁹ PGD₂ is also a neutrophil chemoattractant.

Human mast cells are capable of synthesizing and secreting an array of cytokines. The cytokines appear to vary on conditions of culture, type of disease, and degree and type of stimulus. There are, however, a few generalizations that can be drawn. TNF- α , according to all studies, appears to be a major cytokine produced by human mast cells. It appears to be both stored and synthesized after mast-cell activation. TNF- α upregulates endothelial and epithelial adhesion molecules, increases bronchial responsiveness, and exerts antitumor effects. Other cytokines reported to be produced by human mast cells include IL-4, associated with T_H2 cell differentiation and IgE synthesis; IL-3, GM-CSF, and IL-5, critical for eosinophil development and survival; and IL-6, IL-8, and IL-16.^{13,20} Human mast cells are also documented to produce chemokines, such as macrophage inflammatory protein-1 α .

Roles in health and disease

Mast-cell activation through IgE-dependent mechanisms initiates a cascade of events, resulting in an immediate hypersensitivity reaction, as well as a late-phase reaction (Fig 2). The immediate reaction is reflected in the skin as erythema, edema, and itch; in the upper airways as sneezing, rhinorrhea, and mucous secretion; in the lungs as cough, bronchospasm, edema, and mucous secretion, and in the gastrointestinal tract as nausea, vomiting, diarrhea, and cramping. It is coincident with histamine liberation and demonstration of production of PGD₂ and LTC₄. This reaction is then often followed 6 to 24 hours later by persistent edema and a leukocytic influx, the late-phase reaction, which is due at least in part to the generation and release of the additional mast cell–derived substances listed previously. In turn, recruited cells contribute additional inflammatory mediators at the cellular level. In the lungs, the late-phase reaction is believed to play a major role in the genesis of persistent asthma and the accompanying inflammation. Mast cells have also been suggested to have some contribution to the eventual downregulation of the allergic response, in that they produce and release IL-1 receptor antagonist, heparin, and other molecules with anti-inflammatory properties.²¹

The role that mast cells play in the genesis of allergic diseases may in some way be a reflection of the development of this cell type as critical in both innate and acquired immunity. Mast cells exhibit properties that allow their categorization as a cell involved in innate immunity, including phagocytosis, activation through pattern-recognition receptors, and location at surfaces that face the external environment. A role in acquired immunity is similarly evidenced by their ability to bind parasite-specific IgE, which then leads to mast-cell acti-

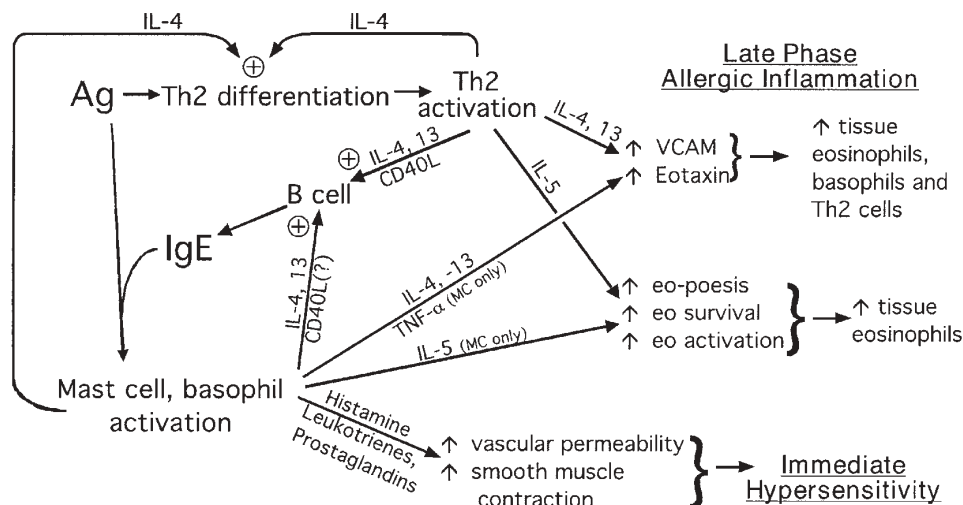


FIG 2. Cellular and molecular mechanisms responsible for allergic responses. Ag, Antigen.

vation after exposure to parasite antigen or the parasite itself. Human mast cells also upregulate Fc γ RI after exposure to IFN- γ . In this case, mast cells could be activated by opsonized infectious organisms. No matter what the activation signal, in every case, mast-cell mediators released and generated would create a protective local inflammatory response.

Pathologic excess of mast cells, usually as a result of activating mutations in the gene for Kit, leads to the disease mastocytosis. This disease can occur in any age group, and in most situations it is first identified because of the appearance of fixed pigmented skin lesions termed *urticaria pigmentosa*. The clinical presentation may also include episodes of unexplained flushing and anaphylaxis. Mastocytosis varies from benign or indolent forms to mastocytosis associated with bone marrow pathology, including myelodysplasia. This disease is usually diagnosed on the basis of characteristic skin findings, an elevated serum level of tryptase, and specific bone marrow findings.²²

BASOPHILS

Basophils are granulocytes that are believed to represent a separate lineage from mast cells, despite the fact that the two cell types share many common features, such as high-affinity IgE receptor (Fc ϵ RI) expression, metachromatic staining, T_H2 cytokine expression, and histamine release. Basophils represent less than 1% of peripheral blood leukocytes, making them one of the least common cell lineages found in the peripheral blood. Peripheral blood basophil numbers are modestly elevated (approximately 2-fold) in allergic asthma.

Morphology and phenotype

Basophils exhibit a segmented nucleus with highly condensed chromatin and are commonly identified by their metachromatic staining with basic dyes, such as toluidine blue. Two basophil granule-specific monoclo-

nal antibodies, BB-1 and 2D7, have recently been developed, permitting the unambiguous identification of basophils in tissues and greatly furthering our understanding of the role of basophils in allergic diseases and asthma.²³ Basophils express a variety of cytokine receptors (IL-3R, IL-5R, GM-CSFR), chemokine receptors (CCR2, CCR3), complement receptors (CD11b, CD11c, CD35, CD88), prostaglandin receptors (CRTH2), and immunoglobulin Fc receptors (Fc ϵ RI, Fc γ RII).^{24,25}

Development

Basophils develop from CD34⁺ pluripotent stem cells, differentiate and mature in the bone marrow, and then circulate in the periphery. IL-3 is the dominant cytokine driving basophil differentiation and is sufficient to differentiate stem cells into basophils.²⁶ The general consensus is that basophils represent a separate cell lineage from mast cells and differentiate from a common basophil-eosinophil precursor; this belief is supported by the derivation of mixed colonies of basophils and eosinophils from individual precursor cells.²⁷

Activation

As with mast cells, basophils express a complete and functional Fc ϵ RI receptor ($\alpha\beta\gamma_2$), cross-linking of which leads to basophil activation, granule exocytosis, and mediator release.²⁸ C3a and C5a can also activate basophils through the C3aR and C5aR complement receptors, respectively. Activation through any of these receptors leads to histamine release, eicosanoid synthesis, and IL-4 and IL-13 gene expression (Fig 2). Priming is the capacity for molecules that cannot maximally activate basophils on their own to augment Fc ϵ RI-mediated activation.²⁹ Mediators with such priming activity include CC chemokines (eotaxins, monocyte chemoattractant protein 3, monocyte chemoattractant protein 4, RANTES), N-formyl-methionyl-leucyl-phenylalanine, IL-3, IL-5, GM-CSF, and histamine-releasing factor.²⁵

The presence of such mediators at sites of allergen exposure may lower the threshold for the development of allergic inflammation.

Mediators

Basophils produce many mediators, similar to mast cells, such as histamine, leukotrienes, IL-4, and IL-13.³⁰ Conversely, the mast-cell mediators PGD₂ and IL-5 are not produced by basophils. Of the newly synthesized eicosanoid mediators, basophils primarily generate LTC₄. In addition to histamine, basophil granules contain a number of other preformed mediators, such as chondroitin sulfate, major basic protein, and Charcot-Leyden crystal protein. Typically, basophils contain only small amounts of tryptase; however, there appears to be a great deal of variation among individuals with regard to basophil tryptase expression.^{31,32}

In addition to their role in immediate hypersensitivity, basophils may contribute to allergic inflammation through a number of nonclassic mechanisms. Basophil expression of IL-4 and CD40L induces B-cell IgE switching *in vitro* and may comprise an alternative mechanism promoting IgE class switching. Alternatively, the rapid and abundant expression of IL-4 by basophils has been suggested as a source of IL-4, which could further drive T_H2 cell differentiation.

Roles in health and disease

The physiologic role of basophils is not known, although presumably, like other leukocytes, they fulfill a role in host defense. Basophils have long been thought to play a role in rejection of ticks and are a conspicuous component of the inflammatory response to many parasites.³³ This proposed role in parasite host defense is further suggested by the recent discovery of functional parasite homologs of histamine-releasing factor in the translationally controlled tumor protein family.^{34,35} Although basophils possess many characteristics that suggest they contribute to allergic inflammation, the precise role of basophils in asthma pathogenesis is unclear. After allergen challenge, basophils are the predominant IL-4-expressing cell type in human asthmatic airways,³⁶ peripheral blood mononuclear cells,³⁷ and a mouse model of asthma.³⁸ By means of basophil-specific monoclonal antibodies, basophils have been identified in cutaneous³⁹ and pulmonary⁴⁰ late-phase responses and are found in increased numbers in the lungs after fatal asthma episodes.⁴¹

EOSINOPHILS

Eosinophils are granulocytes that were first described to stain with acid aniline dyes, such as eosin. Although these cells are rare in the peripheral blood of healthy persons, blood and tissue eosinophilia are hallmark signs of helminth infection, allergy, and asthma. Because of the large body of evidence supporting a critical role in asthma pathogenesis, the eosinophil has emerged as a major therapeutic target for immunologically based asthma therapies.⁴²⁻⁴⁴

Morphology and phenotype

Eosinophils typically exhibit a bilobar nucleus with highly condensed chromatin and cytoplasm containing two major types of granules, specific and primary granules. Specific granules have a distinctive ultrastructural appearance, consisting of an electron-dense crystalloid core. These granules contain the many cationic proteins that give eosinophils their unique staining properties. Primary granules are similar to those found in other granulocyte lineages and are found early in eosinophil development.⁴⁵ Eosinophils also contain lipid bodies, which play a role in the generation of eicosanoid mediators.⁴⁶

Because there is no eosinophil-specific cell-surface marker, their binding of eosinlike dyes remains the most common detection method. The development of monoclonal antibodies to the various eosinophil granule proteins has emerged as an additional means of identifying these cells immunochemically.^{47,48} Eosinophils express a wide array of cell-surface molecules, including cytokine receptors (IL-3R, IL-5R, and GM-CSFR), chemokine receptors (CCR1 and CCR3), FcγRII (CD32), FcαRI (secretory IgA), complement receptors (C3aR, C5aR, CD88, and CD35), adhesion molecules (very late antigen [VLA]-4 and α₄β₇ integrin), CD9, and CD69.^{25,49} CD69 is an activation marker for eosinophils and is upregulated on eosinophils isolated from sites of allergic inflammation.²⁹ Eosinophil expression of FcεRI is minimal and is of unclear functional significance.^{50,51}

Development and trafficking

Eosinophils develop and mature in the bone marrow from CD34⁺ progenitor cells and are released to the peripheral blood as mature cells. IL-5, the major eosinophil-active cytokine, has a profound positive effect on the differentiation and proliferation of eosinophil precursors in the bone marrow.^{42,45} In this manner, IL-5 produced peripherally at sites of allergic inflammation, or helminth infection acts distally on the bone marrow. Additionally, allergen challenge or the experimental administration of eotaxin causes bone marrow release of mature eosinophils, as well as eosinophil precursors.⁵²

Once released from the bone marrow, eosinophils circulate in the peripheral blood and then traffic to tissue with a peripheral blood half-life of between 8 and 18 hours. Although eosinophils are best known as peripheral blood leukocytes, the vast majority of eosinophils are located in the gut and lungs.⁵³ The steps required for eosinophil trafficking from the peripheral circulation to tissue have been well characterized,²⁵ and there is currently a great emphasis on exploiting these mechanisms to treat asthma. After selectin and VLA-4-mediated rolling, firm adhesion is mediated largely through the interaction of eosinophil VLA-4 with endothelial cell-expressed vascular cell adhesion molecule (VCAM)-1. Eotaxin and other CC chemokines serve both to increase the avidity of the VLA-4 molecule for VCAM-1 and to directly promote eosinophil chemotaxis through the tissue.⁵⁴ IL-4 and IL-13 play central roles in promoting eosinophil trafficking

by increasing endothelial cell VCAM-1 expression and upregulating eotaxin expression by bronchial epithelial cells and fibroblasts. TNF- α acts synergistically with IL-4 and IL-13 to promote VCAM-1 expression. Two additional eotaxin homologs, eotaxin 2 and eotaxin 3, have recently been identified, suggesting additional complexity in this system. In contrast to eotaxin, IL-5 does not play a major role in promoting eosinophil entry into tissues. In addition to eotaxin, platelet-activating factor (PAF) and LTB₄ are also potent eosinophil chemotactic factors.

Activation

Once they have trafficked to the tissue, leukocytes need an activation signal to exhibit their effector function. In contrast to Fc ϵ RI activation of mast cells and basophils, there is no consensus regarding the major physiologic mechanism of eosinophil activation. Eosinophils are activated by cross-linking of beads coated with either IgG, IgA, or secretory IgA, the last being the most potent.²⁹ These results, and the prominent location of eosinophils in the gut and lungs, suggest that eosinophils may play a role in host defense surveillance of mucosal surfaces. Eosinophils from donors with peripheral eosinophilia can be activated with anti-IgE or IgE-coated parasites. However, most reports have not demonstrated expression of functional Fc ϵ RI by eosinophils.^{50,51}

Eosinophils also show the capacity to be primed by a number of mediators, including IL-3, IL-5, GM-CSF, CC chemokines, and PAF. Eosinophils obtained from bronchoalveolar lavage after allergen challenge demonstrate a primed phenotype, supporting the *in vivo* relevance of this priming phenomenon. IL-5, GM-CSF, and, to a lesser extent, IL-3 have an antiapoptotic effect on eosinophils and promote eosinophil survival in tissues. Activated or primed eosinophils often exhibit lower density than resting eosinophils and are termed *hypodense*.²⁹

Mediators and effector function

Eosinophils release a variety of proinflammatory mediators, including granule-stored cationic proteins, newly synthesized eicosanoids, and cytokines.⁴⁵ Eosinophils contain a wide array of highly basic (isoelectric point pH 9-11) granule proteins that appear to play a role both in host defense and in the pathogenesis of eosinophil-mediated diseases.

Major basic protein (MBP) is so named because it accounts for over 50% of the eosinophil granule protein mass. As a purified protein, MBP has potent *in vitro* toxicity against a number of parasites, including helminths and schistosomes.⁵⁵ MBP is directly toxic to respiratory epithelial cells and causes bronchial hyperresponsiveness and bronchoconstriction when instilled into the lungs of cynomolgus monkeys. This finding and the correlation of serum and bronchoalveolar lavage MBP levels with bronchial hyperresponsiveness suggest that MBP is a major effector molecule in asthma pathogenesis.⁴²

Eosinophil-derived neurotoxin (EDN) is so named because it is toxic to myelinated neurons when injected into experimental animals. Some of the neurologic man-

ifestations of the hypereosinophilic syndrome may be mediated through this mechanism. Both EDN and eosinophil cationic protein demonstrate RNase activity and are capable of killing single-stranded RNA pneumoviruses, such as respiratory syncytial virus. The EDN and eosinophil cationic protein genes show exceedingly high rates of molecular evolution, suggesting that the molecules are under extraordinary selective pressure, as might be expected of genes responding to the rapid evolution of microbial pathogens.^{42,56} Additionally, EDN and eosinophil cationic protein demonstrate *in vitro* toxicity to parasites.⁵⁵ Eosinophil peroxidase has homology with neutrophil myeloperoxidase and is capable of generating microbiocidal hypohalous acids.⁴⁵

Eosinophils are also a major source of the cysteinyl leukotriene LTC₄ and its active metabolites LTD₄ and LTE₄. Eosinophils, along with mast cells and basophils, are the principal LTC₄-synthase-producing cell in asthmatic bronchial mucosa.⁴⁶ Eosinophils are capable of producing a number of cytokines, including IL-1, transforming growth factor β , IL-3, IL-4, IL-5, IL-8, and TNF- α . However, eosinophils generally produce lower amounts of cytokines than do other inflammatory cells, such as T cells.^{29,44} As such, the relative contribution of eosinophil cytokine production to the generation of allergic inflammation remains to be determined.

Roles in health and disease

Peripheral blood eosinophil counts are increased in allergic disease, asthma, and helminth infections, and tissue eosinophilia is often found at inflammatory sites associated with these diseases.⁵⁷ Despite their *in vitro* activity against parasites, *in vivo* studies in IL-5 gene knockout mice have generally not supported an essential role for eosinophils in clearing parasitic infections.⁵⁵

In allergic disease and asthma, eosinophils play a proinflammatory role, in which eosinophil mediators, such as MBP are thought to cause mucosal inflammation and consequent bronchial hyperresponsiveness.⁴² Corticosteroids potently decrease both peripheral blood and tissue eosinophil counts, further supporting a central role for eosinophils in asthma pathogenesis. Because eosinophils have been considered the end effector cell of asthma, a number of investigational therapies have targeted eosinophils. Recently, a phase II study of anti-IL-5 in human asthma demonstrated no improvement in either airflow or late-phase allergic responses despite a 90% decrease in peripheral blood eosinophils.⁴³ A subsequent study of anti-IL-5 demonstrated a similar 90% drop in peripheral blood eosinophil counts but only a 55% drop in bronchial mucosal eosinophils. These data demonstrate that anti-IL-5 alone may not be sufficient to abrogate pulmonary eosinophilia and that additional antieosinophil strategies must be examined to determine their therapeutic potential in the treatment of asthma.

REFERENCES

1. Hamilton RG. Assessment of human allergic disease. In: Rich RR, Fleisher TA, Shearer WT, Katzin BL, Schroeder HW, editors. Clinical

- immunology: principles and practice. 2nd ed. London: Mosby; 2001. p. 124.1-14.
2. Ownby DR. Clinical significance of immunoglobulin E. In: Middleton E, Reed CE, Ellis EF, Adkinson NF, Yunginger JW, Busse WW, editors. *Allergy: principles and practice*. 5th ed. St Louis: Mosby; 1998. p. 770-82.
3. Verelli D. The regulation of IgE synthesis. *Clin Allergy Immunol* 2002;16:179-96.
4. Oettgen HC, Geha RS. IgE regulation and roles in asthma pathogenesis. *J Allergy Clin Immunol* 2001;107:429-40.
5. Gould HJ, Beavil RL, Vercelli D. IgE isotype determination: ϵ -germline gene transcription, DNA recombination and B-cell differentiation. *Br Med Bull* 2000;56:908-24.
6. Adra CN, Gao PS, Mao XQ, Baron BW, Pauker S, Miki T, et al. Variants of B cell lymphoma 6 (RCL6) and marked atopy. *Clin Genet* 1998; 54:362-4.
7. Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S, Zurawski G, et al. Interleukin 13 induces interleukin 4-independent IgG₄ and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci U S A* 1993;90:3730-4.
8. Saini SS, Klion AD, Holland SM, Hamilton RG, Bochner BS, MacGlashan DW. The relationship between serum IgE and surface levels of Fc ϵ R on human leukocytes in various diseases: correlation of expression of Fc ϵ R1 on basophils but not on monocytes or eosinophils. *J Allergy Clin Immunol* 2000;106:514-20.
9. Tkaczuk C, Gilfillan AM. Fc ϵ R1-dependent signaling pathways in human mast cells. *Clin Immunol* 2001;99:198-210.
10. Kopley CL, Youssef L, Andrews RP, Wilson BS, Oliver JM. Multiple defects in Fc ϵ R1 signaling in Syk-deficient nonreleaser basophils and IL-3 induced recovery of Syk expression and secretion. *J Immunol* 2000;165:5913-20.
11. Kopley CL, Cambier JC, Morel PA, Lujan D, Ortega E, Wilson BS, et al. Negative regulation of Fc ϵ R2 costimulation in human blood basophils. *J Allergy Clin Immunol* 2000;106:337-48.
12. Taylor ML, Metcalfe DD. Mast cells in allergy and host defense. *Allergy Asthma Proc* 2001;22:115-9.
13. Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev* 1997;77:1033-79.
14. Schwartz LB. Mast cells and basophils. *Clin Allergy Immunol* 2002;16:3-42.
15. Quackenbush EJ, Wershil BK, Gutierrez-Ramos JC. Modulation of mast cell development from embryonic haematopoietic progenitors by eotaxin. In: Marone G, Lichtenstein LM, Galli SJ, editors. *Mast cells and basophils*. London: Academic Press; 2000. p. 31-49.
16. Galli SJ, Metcalfe DD, Dvorak AM. Basophils and mast cells and their disorders. In: Beutler E, Lichtman MA, Collier BS, Kipps TJ, Seligsohn U, editors. *Williams hematology*. 6th ed. New York: McGraw-Hill; 2001. p. 801-15.
17. Nilsson GM, Johnell CH, Hammer HL, Tiffany HL, Nilsson K, Metcalfe DD, et al. C3a and C5a are chemotaxins for human mast cells and act through distinct receptors via a pertussis toxin sensitive signal transduction pathway. *J Immunol* 1996;157:1693-8.
18. Nilsson G, Forsberg-Nilsson K, Hallbook F, Nilsson K, Metcalfe DD. Human mast cells express functional TRKA and are a source of nerve growth factor. *Eur J Immunol* 1997;27:2295-301.
19. Tkaczuk C, Okayama Y, Woolhiser M, Hagaman D, Gilfillan A, Metcalfe D. Activation of human mast cells through the high affinity IgG receptor. *Mol Immunol* 2002;38:1289.
20. Church MK, Holgate ST, Shute JK, Walls AF, Sampson AP. Mast cell derived mediators. In: Middleton E, Reed CE, Ellis EF, Adkinson NF, Yunginger JW, Busse WW, editors. *Allergy principles and practice*. 5th ed. St Louis: Mosby; 1998. p. 146-67.
21. Hagaman DD, Okayama Y, D'Ambrosio C, Gilfillan AM, Prussin C, Metcalfe DD. Secretion of interleukin-1 receptor antagonist from human mast cells following IgE mediated activation and following segmental antigen challenge. *Am J Respir Cell Mol Biol* 2001;25:685-91.
22. Simpson JK, Metcalfe DD. Mastocytosis and disorders of mast cell proliferation. *Clin Rev Allergy Immunol* 2002;22:175-88.
23. Buckley MG, McEuen AR, Walls AF. The return of the basophil. *Clin Exp Allergy* 2002;32:8-10.
24. Agis H, Fureder W, Bankl HC, Kundi M, Speer WR, Willheim M, et al. Comparative immunophenotypic analysis of human mast cells, blood basophils and monocytes. *Immunology* 1996;87:535-43.
25. Bochner BS, Schleimer RP. Mast cells, basophils, and eosinophils: distinct but overlapping pathways for recruitment. *Immunol Rev* 2001;179:5-15.
26. Kopley CL, Pfeiffer JR, Schwartz LB, Wilson BS, Oliver JM. The identification and characterization of umbilical cord blood-derived human basophils. *J Leukoc Biol* 1998;64:474-83.
27. Denburg JA, Telizyn S, Messner H, Lim B, Jamal N, Ackerman SJ, et al. Heterogeneity of human peripheral blood eosinophil-type colonies: evidence for a common basophil-eosinophil progenitor. *Blood* 1985;66:312-8.
28. Kinet JP. The high-affinity IgE receptor (Fc ϵ R1): from physiology to pathology. *Annu Rev Immunol* 1999;17:931-72.
29. Bochner BS. Systemic activation of basophils and eosinophils: markers and consequences. *J Allergy Clin Immunol* 2000;106(5 Suppl):S292-302.
30. Schroeder JT, MacGlashan DW Jr, Lichtenstein LM. Human basophils: mediator release and cytokine production. *Adv Immunol* 2001; 77:93-122.
31. Li L, Li Y, Reddel SW, Cherrian M, Friend DS, Stevens RL, et al. Identification of basophilic cells that express mast cell granule proteases in the peripheral blood of asthma, allergy, and drug-reactive patients. *J Immunol* 1998;161:5079-86.
32. Foster B, Schwartz LB, Devouassoux G, Metcalfe DD, Prussin C. Characterization of mast-cell tryptase-expressing peripheral blood cells as basophils. *J Allergy Clin Immunol* 2002;109:287-93.
33. Falcone FH, Pritchard DI, Gibbs BF. Do basophils play a role in immunity against parasites? *Trends Parasitol* 2001;17:126-9.
34. Rao KV, Chen L, Gnanasekar M, Ramaswamy K. Cloning and characterization of a calcium-binding, histamine-releasing protein from *Schistosoma mansoni*. *J Biol Chem* 2002;277:31207-13.
35. MacDonald SM, Bhisutthibhan J, Shapiro TA, Rogerson, SJ, Taylor TE, Tembo M, et al. Immune mimicry in malaria: *Plasmodium falciparum* secretes a functional histamine-releasing factor homolog in vitro and in vivo. *Proc Natl Acad Sci U S A* 2001;98:10829-32.
36. Nouri-Aria KT, Irani AM, Jacobson MR, O'Brien F, Varga EM, Till SJ, et al. Basophil recruitment and IL-4 production during human asthmatic allergen-induced late asthma. *J Allergy Clin Immunol* 2001;108:205-11.
37. Devouassoux G, Foster B, Scott LM, Metcalfe DD, Prussin C. Frequency and characterization of antigen-specific IL-4- and IL-13-producing basophils and T cells in peripheral blood of healthy and asthmatic subjects. *J Allergy Clin Immunol* 1999;104:811-9.
38. Lucciolli S, Brody DT, Hasan D, Kojima N, Noben-Trauth N, et al. IgE⁺ Kit I-A/I-E myeloid cells are the initial source of IL-4 following antigen challenge in a mouse model of allergic pulmonary inflammation. *J Allergy Clin Immunol* 2002;110:117-24.
39. Gauvreau GM, Lee JM, Watson RM, Irani AM, Schwartz LB, O'Byrne PM. Increased numbers of both airway basophils and mast cells in sputum after allergen inhalation challenge of atopic asthmatics. *Am J Respir Crit Care Med* 2000;161:1473-8.
40. Macfarlane AJ, Kon OM, Smith SJ, Zeibecoglou K, Khan LN, Barata LT, et al. Basophils, eosinophils, and mast cells in atopic and nonatopic asthma and in late-phase allergic reactions in the lung and skin. *J Allergy Clin Immunol* 2000;105:99-107.
41. Kopley CL, McFeeley PJ, Oliver JM, Lipscomb MF. Immunohistochemical detection of human basophils in postmortem cases of fatal asthma. *Am J Respir Crit Care Med* 2001;164:1053-8.
42. Gleich GJ. Mechanisms of eosinophil-associated inflammation. *J Allergy Clin Immunol* 2000;105:651-63.
43. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2144-8.
44. Lee NA, Gelfand EW, Lee JJ. Pulmonary T cells and eosinophils: coconspirators or independent triggers of allergic respiratory pathology? *J Allergy Clin Immunol* 2001;107:945-57.
45. Kita H, Adolphson C, Gleich G. Biology of eosinophils. In: Middleton E, editor. *Allergy principles and practice*, vol 1. 5th ed. St Louis: Mosby; 1998. p. 242-60.
46. Bandeira-Melo C, Bozza PT, Weller PF. The cellular biology of eosinophil eicosanoid formation and function. *J Allergy Clin Immunol* 2002;109:393-400.
47. Krug N, Thureau AM, Lackie P, Baier J, Schultze-Werninghaus G, Rieger CH, et al. A flow cytometric method for the detection of intracellular basic proteins in unseparated peripheral blood and bone marrow eosinophils. *J Immunol Methods* 1996;190:245-54.

48. Ying S, Meng Q, Smith SJ, Larche M, Robinson DS, Kay AB. Methods for identifying human eosinophils in blood and tissue. *ACI Int* 2002;12:64-71.
49. Tachimoto H, Bochner BS. The surface phenotype of human eosinophils. *Chem Immunol* 2000;76:45-62.
50. Seminario MC, Saini SS, MacGlashan DW Jr, Bochner BS. Intracellular expression and release of FcεRI α by human eosinophils. *J Immunol* 1999;162:6893-900.
51. Kita H, Kaneko M, Bartemes KR, Weiler DA, Schimming AW, Reed CE, et al. Does IgE bind to and activate eosinophils from patients with allergy? *J Immunol* 1999;162:6901-11.
52. Denburg JA, Sehmi R, Saito H, Pil-Seob J, Inman MD, O'Byrne PM. Systemic aspects of allergic disease: bone marrow responses. *J Allergy Clin Immunol* 2000;106(5 Suppl):S242-6.
53. Hogan SP, Foster PS, Rothenberg ME. Experimental analysis of eosinophil-associated gastrointestinal diseases. *Curr Opin Allergy Clin Immunol* 2002;2:239-48.
54. Lukacs NW. Role of chemokines in the pathogenesis of asthma. *Nat Rev Immunol* 2001;1:108-16.
55. Meeusen EN, Balic A. Do eosinophils have a role in the killing of helminth parasites? *Parasitol Today* 2000;16:95-101.
56. Rosenberg HF, Domachowske JB. Eosinophils, eosinophil ribonucleases, and their role in host defense against respiratory virus pathogens. *J Leukoc Biol* 2001;70:691-8.
57. Rothenberg ME. Eosinophilia. *N Engl J Med* 1998;338:1592-600.