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Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils

Stephen J Galli[1,2], **Niels Borregaard**[3], and **Thomas A Wynn**[4]

[1]Department of Pathology, Stanford University School of Medicine, Stanford, California, USA

[2]Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, California, USA

[3]Department of Hematology, National University Hospital, University of Copenhagen, Copenhagen, Denmark

[4]Program in Barrier Immunity and Repair, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA

Abstract

Hematopoietic cells, including lymphoid and myeloid cells, can develop into phenotypically distinct ‘subpopulations’ with different functions. However, evidence indicates that some of these subpopulations can manifest substantial plasticity (that is, undergo changes in their phenotype and function). Here we focus on the occurrence of phenotypically distinct subpopulations in three lineages of myeloid cells with important roles in innate and acquired immunity: macrophages, mast cells and neutrophils. Cytokine signals, epigenetic modifications and other microenvironmental factors can substantially and, in some cases, rapidly and reversibly alter the phenotype of these cells and influence their function. This suggests that regulation of the phenotype and function of differentiated hematopoietic cells by microenvironmental factors, including those generated during immune responses, represents a common mechanism for modulating innate or adaptive immunity.

In innate or adaptive immunity, differentiated hematopoietic cells must orchestrate complex functional programs to promote host defense while also limiting maladaptive collateral damage to the tissues in which such responses take place. There is mounting evidence that one of the mechanisms used to achieve this goal is the induction of alterations in the phenotype of various cells of the innate or adaptive immunity, which positions them to serve the appropriate functions in distinct contexts. However, the extent of such ‘functional plasticity’ of differentiated hematopoietic cells and the mechanisms that regulate such phenotypic changes remain to be fully understood.

In principle, substantial changes in cell phenotype and function can be achieved by exposure of susceptible differentiated cell populations to the appropriate mixtures of a relatively small number of signals. In the example of induced pluripotent stem cells, enforced expression of a limited set of transcription factors permits adult somatic cells to gain features of pluripotency, which in turn permits the directed differentiation of such induced pluripotent

Correspondence should be addressed to S.J.G. (sgalli@stanford.edu), N.B. (borregaard@rh.dk) or T.A.W. (twynn@niaid.nih.gov).

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stem cells into various new cell lineages with highly specialized functions[1]. The direct reprogramming of adult somatic cells into those with features of another distinct cell type also has been achieved[1].

Although the hematopoietic system has substantial developmental plasticity, as exemplified by the ability of hematopoietic stem cells to give rise to all hematopoietic lineages[2,3], the ability of the main differentiated hematopoietic cell types to undergo substantial phenotypic changes is thought to be much more limited. However, some of these differentiated cell types (for example, T cells[4], B cells[5] and dendritic cells (DCs)[6]) consist of phenotypically distinct subpopulations that can serve different functions. In such settings, it has become customary to define and then name such subpopulations in part on the basis of their phenotypic characteristics and in part on the basis of their actions and then, when analyzing such cells *in vitro* or *in vivo*, to use such phenotypic features to infer function. This must be done cautiously, as some hematopoietic cells can have surface structures and other features of more than one 'classical' cell lineage, such as macrophages and DCs[7] or DCs and B cells[8].

Moreover, published work has called into question the stability of certain subpopulations of hematopoietic cells. For example, some T cell subpopulations can show considerable plasticity, at least under *in vitro* conditions[9–11], including examples of situations in which T cell populations with features of one subset (for example, regulatory T cells) can acquire the phenotypic and functional characteristics of another (for example, interleukin 17 (IL-17)-producing helper T cells (T_H17 cells))[11]. Given such findings, the following general questions might arise: to what extent is the phenotype and function of differentiated hematopoietic cell types intrinsically variable, either at the level of the single cell or the population; how is such variation regulated; and, equally important, what are the implications of such phenotypic plasticity for the roles of such cells in health or disease?

In this review we have addressed those questions in the context of three populations of myeloid cells that function mainly in the peripheral tissues and can have important roles in both innate and adaptive immune responses: macrophages, mast cells and neutrophils. We review the evidence that there are phenotypically and functionally distinct subpopulations or activation phenotypes of these three main lineages of myeloid cells and consider briefly some of what is known about the consequences of such plasticity in the context of the functions of these cell types during innate and adaptive immunity.

Basic biology of macrophages

A committed progenitor cell in the bone marrow is responsible for generating the mononuclear phagocyte system[3]. The two main populations in this lineage are the macrophages and DCs, which display many common cell surface receptors but have distinct functional activities. In contrast to neutrophils, which are short-lived, there is great complexity in the lifespan of macrophages and DCs, which varies from hours to possibly years depending on the nature of the immune response[7]. Tissue macrophages differentiate from circulating monocytes when they enter tissues and are distinguished from DCs by their expression of F4/80, CD11b and Fc receptors; also, in contrast to DCs, which serve as the main inducers of adaptive T cell responses, macrophages have proteolytic and catabolic activities and are more skilled at ingesting pathogens by phagocytosis, scavenging dead cells and cellular debris, and remodeling tissues after injury[12–14] (Table 1).

Macrophages and DCs can be further subcategorized into subpopulations on the basis of their anatomical location and functional phenotype[15] (Fig. 1). Tissue-resident macrophages include osteoclasts (bone), microglia (brain), alveolar macrophages (lung), histiocytes (interstitial connective tissue) and Kupffer cells (liver). There are also many mononuclear

phagocyte subpopulations in the circulation and in the spleen that can differentiate into macrophages[12,16]. Although their phenotypes and names vary on the basis of their anatomical location, they all act like macrophages and acquire similar functional abilities when stimulated appropriately[17].

Because phenotypic markers have not been particularly helpful in distinguishing the many subpopulations of macrophages[13], a more useful approach has been to define macrophages on the basis of their specific functional activities[18]. Cells with a variety of functional phenotypes have been described, including classically activated macrophages (M1 macrophages; which mediate host defense and antitumor immunity), alternatively activated macrophages (M2 macrophages; which are suppressors and regulate wound healing), regulatory macrophages (which secrete IL-10), tumor-associated macrophages (which suppress tumor immunity) and the monocytic subset of myeloid-derived suppressor cells (MDSCs; which are functionally similar to tumor-associated macrophages), to name just a few[19]. Although there are some differences among the M2, regulatory, tumor-associated and MDSC subsets, each of these populations has mainly immunosuppressive activity[17]. Consequently, macrophages have one of two major phenotypes: they either induce host defense, antitumor immunity and inflammatory responses or suppress those functions. Therefore, macrophages, like T cells, can have active roles in both the induction and the resolution of immune responses.

Regulation of the phenotype and function of macrophages

Macrophages maintain tissue homeostasis by serving various housekeeping functions that apparently require no special activating stimuli or are activated during ontogeny. Macrophages are phagocytic cells that constitutively express a variety of scavenger receptors that facilitate the removal of aged red blood cells, necrotic tissues and toxic molecules from the circulation[12]. However, to keep up with greater demand, these homeostatic functions are increased by a variety of activating stimuli after tissue injury or during infection. Thus, like DCs, macrophages serve as sentinel cells for the immune response. They express pattern-recognition receptors that identify pathogen-associated or damage-associated molecular patterns expressed by microbial pathogens (for example, lipopolysaccharide) or during cellular stress (for example, nuclear and cytosolic proteins), respectively[12]. These receptors ‘instruct’ macrophages to produce a variety of mediators that recruit neutrophils and promote inflammation[20].

Pathogen-associated and damage-associated molecular patterns also act in synergy with natural killer cell–derived interferon-γ to polarize macrophages toward the M1 phenotype, which is characterized by the production of reactive oxygen and nitrogen species that facilitate the killing of microbial pathogens[19,21]. M2 polarization, in contrast, is a programmed response facilitated by the signal transducer STAT6–activating cytokines IL-4 and IL-13 (ref. 12). Although $CD4^+$ type 2 helper T cells (T_H2 cells) are important inducers of M2 macrophages, a variety of innate IL-4- and IL-13-producing cells, such as basophils, nuocytes and natural helper cells, also may contribute to M2 polarization[22–24]. In addition to IL-4 and IL-13, IL-10, IL-21, the cell-signaling molecule GM-CSF, IL-33 and unique transcription factors regulate the differentiation of M2 cells[25,26].

Epigenetic changes regulated by Jmjd3, a demethylase of histone 3 Lys27 (H3K27), have also been shown to control IL-4-induced M2 polarization[27]. IL-4 activates Jmjd3 expression, which results in less H3K27-dimethylation and H3K27-trimethylation marks in the promoters of several M2 marker genes, including *Chi3l3*, *Retnla* and *Arg1*, leading to their transcriptional activation[28]. It also induces expression of the transcription factor IRF4, which promotes M2 polarization while inhibiting the transcription of many M1-associated

genes[26]. A role for $CD4^+CD25^+Foxp3^+$ regulatory T cells in the polarization of human M2-like macrophages has also been described[29]. In contrast to the proinflammatory M1 cells, M2 macrophages suppress inflammation and antitumor immunity, facilitate wound repair, regulate glucose metabolism and mediate the expulsion of extracellular parasites from the gut[18,30,31].

A third main class of macrophages called 'regulatory macrophages', similar to suppressive M2 cells, have also been described[19]. These macrophages are induced by Toll-like receptor (TLR) agonists in the presence of immunoglobulin G (IgG) immune complexes, apop-totic cells and prostaglandins and are defined by production of the immunosuppressive cytokines IL-10 and TGF-β1 (ref. 19). These cells are poor antigen-presenting cells and have a propensity to induce T_H2 and regulatory T cell responses, which can further suppress chronic inflammatory and antitumor responses. Although distinct subpopulations of macrophages with unique functional abilities have been described, it is generally thought that macrophages represent a spectrum of activated phenotypes rather than discrete stable subpopulations[19]. Indeed, many studies have documented flexibility in macrophage programming, with macrophages readily switching from one functional phenotype to another in response to new microenvironmental signals[32–35].

Macrophages in innate immunity

Macrophages are cells that function in both innate and adaptive immunity and can exert protective and pathogenic activity. They express a variety of pattern-recognition receptors, including TLRs, C-type lectin receptors, helicase RIG–like receptors and biosensor Nod–like receptors that recognize danger signals associated with invading pathogens, foreign substances (for example, silica and asbestos), and dead or dying cells[7,12]. These receptors all have important roles in the activation of the innate immune response. They 'instruct' macrophages to engulf and destroy foreign particles and bacteria through the generation of a respiratory burst, thus promoting an M1-like phenotype. They also activate signaling by the adaptor MyD88 and regulate inflammasome formation[19,36], which can enhance the innate antimicrobial activity of M1 cells by stimulating the production of cytokines such as TNF and IL-1β.

In addition to their innate phagocytic activity and role in antimicrobial immunity, macrophages are also intimately involved in wound repair[14]. Macrophages are rapidly recruited to wounds after platelet degranulation, which may reflect in part the response of macrophages to platelet-derived growth factor[37], and macrophages can secrete a wide variety of cytokines and chemokines, as well as matrix metalloproteinases and their tissue inhibitors, that regulate the recruitment of cells to and deposition of extracellular matrix components at sites of tissue injury[38]. Macrophages with a wound-healing M2-like phenotype also regulate important metabolic functions[39]. These macrophages are induced by signaling via the receptor PPAR-γ and maintain adipocyte function, insulin sensitivity and glucose tolerance, which prevents the development of diet-induced obesity[40,41]. It has been reported that IL-4-producing eosinophils are needed to maintain this particular macrophage population[42]. Those studies suggest that as obesity progresses, adipose-associated macrophages switch from an M2-like phenotype to a classically activated M1-like cell with potent proinflammatory activity[39], with the NLRP3 inflammasome serving as the molecular switch by sensing obesity-associated danger signals[43].

Macrophages in adaptive immunity

Although they are not as efficient as DCs, macrophages function as antigen-presenting cells and participate in the activation of the adaptive arm of the immune response[12]. Natural killer cells often provide the initial interferon-γ that 'instructs' macrophages to develop a

classically activated M1 phenotype[19]. These innate inflammatory macrophages produce large amounts of TNF, IL-12 and IL-23 and therefore are important drivers of antigen-specific type 1 helper T cell (T_H1 cell) and T_H17 cell responses[44]. T cell–derived interferon-γ in turn feeds back, providing a positive amplification loop that expands the population of M1 cells while also increasing their microbicidal and tumoricidal activity[19]. M1 cells are generally believed to serve a protective role in tumorigenesis by antagonizing the suppressive activities of tumor-associated macrophages, MDSCs, alternatively activated macrophages and regulatory macrophages, which promote tumor growth, invasion and metastasis by suppressing adaptive antitumor immune responses[45]. Because M1 macrophages secrete large amounts of TNF and IL-1β and participate in the differentiation of T_H17 cells, they are also believed to be important drivers of chronic inflammatory and autoimmune diseases, including rheumatoid arthritis, atherosclerosis, pulmonary fibrosis and Crohn's disease[46–48].

In contrast to M1 cells, M2 cells have mainly suppressive or immunoregulatory activity. They antagonize toxic M1 responses, dampen inflammation, suppress antitumor immunity and promote wound healing, tissue remodeling and angiogenesis[12,49]. Regulatory macrophages that secrete IL-10 have similar roles in adaptive immune responses, although they are particularly adept at suppressing antimicrobial immunity[19]. Regulatory macrophages also facilitate the maintenance of immune homeostasis in the gut by inducing the development of regulatory T cells[50], whereas M2 cells mediate secondary immunity to gastrointestinal worms[30]. Although alternatively activated macrophages are induced by a variety of innate IL-4- and IL-13-producing cells, including basophils[51], T_H2 cells are thought to serve as the main inducers of M2 cells when the adaptive immune response is activated, as in many chronic inflammatory and fibrotic diseases[14,18,31].

Basic biology of mast cells

Mast cells are derived from hematopoietic stem cells but, as is true for macrophages, the mature cells do not ordinarily circulate[52] (Table 1). Instead, the precursor cells differentiate and mature locally after their migration into the vascularized tissues or (in rodents) serosal cavities in which mast cells will ultimately reside[52]. In vertebrates, mast cells are widely distributed throughout tissues, especially near surfaces exposed to the environment (for example, skin, airways and the gastrointestinal and genitourinary tracts), where pathogens, allergens and other environmental agents are frequently encountered[52]. This distribution permits them, along with DCs and tissue macrophages, to be among the first cells of the immune response to interact with environmental antigens and allergens, invading pathogens or environmentally derived toxins[53]. Like macrophages, mast cells can be long-lived, and at least some tissue mast cells can proliferate after the appropriate stimulation[52,54]. Although the extent to which various macrophage subpopulations have proliferative ability remains controversial, it has been reported that M2 macrophages can proliferate[55]. Mast cell populations also can expand via enhanced recruitment, survival and maturation of progenitors[52,54]. Many innate or acquired immune responses, including those associated with T_H2-type responses, and many diseases processes are associated with changes in number of mast cells at the affected sites[52,54].

Although mast cells share many structures and functions with macrophages (including many pathogen-associated and damage-associated molecular patterns) and have been reported to perform antigen presentation and phagocytosis[56], mast cells are particularly specialized to serve functions that can amplify or suppress innate or acquired immune responses[52,53,56–61]. In large part, such functions reflect the ability of mast cells to secrete a wide spectrum of preformed or newly synthesized biologically active products, many of which can potentially mediate proinflammatory, anti-inflammatory and/or immunosuppressive

functions[52,54,56,58,59] (Fig. 2). Mast cells can participate in many cycles of activation for the release of mediators and can be activated to release distinct patterns of mediators or cytokines depending on the type and strength of the activating stimuli[52,54,57,62] (Fig. 2). Moreover, the strength and nature of the response of mast cells to various activating stimuli, including aggregation of surface high-affinity IgE receptors (FcεRI receptors) by IgE and specific antigen (the main stimulus of mast-cell activation in allergic disorders and immune responses to parasites), may be influenced by genetic or microenvironmental factors that affect the expression pattern or functional properties of the surface receptors or signaling molecules that contribute to such responses[52,54,57,62].

Regulation of the phenotype and function of mast cells

Environmental and genetic factors can finely control or 'tune' many key characteristics of mast-cell populations, such as their proliferation, survival and ability to store and/or produce various secreted products, and the magnitude and nature of their secretory responses to specific stimuli of activation generated during innate or acquired immune responses[52]. Stem-cell factor (SCF; also known as the ligand for the receptor c-Kit (CD117)) is the main survival and developmental factor for mast cells, but many growth factors, cytokines and chemokines can influence the number and mediator content of mast cells and other aspects of their phenotype, including IL-3, which is especially important in mice, T_H2-associated cytokines (such as IL-4 and IL-9), and TGF-β_1 (refs. 52, 54).

Important aspects of mast-cell phenotype can vary according to animal species, anatomical location, individual genetics or strain background, systemic or local changes in the amount of factors that can alter various properties of the cell, and whether the cell is analyzed *in vivo* or *in vitro*[52,63] (Fig. 2). As the main functions of mast cells are thought to reflect the biological activities of their secreted products, it is widely thought that factors that influence either the spectrum of stimuli that can activate the release of mast-cell mediators (such as the types of immunoglobulins or pattern-recognition receptors expressed by the cells) or the ability of the cells to produce various mediators will in turn alter the functions of the cells *in vivo*[52,54,56,58,59]

Mast cells in innate immunity

Like macrophages, mast cells also can function in both innate and adaptive immune responses and can have protective and pathogenic activity. A chief role of mast cells in innate immunity is to enhance the local recruitment of neutrophils, a function that can either enhance host resistance or contribute to pathology[56,64,65]. Although there are many examples of how changes in mast-cell phenotype might influence the function of the cells of innate immunity, variation in the ability of mast cells to synthesize and store different proteases or proteoglycans is of particular interest. Stored serine proteases and proteoglycans represent a substantial fraction of the mass of a mast cell, and individual mast-cell subpopulations can store different mixtures of such proteases and proteoglycans (Fig. 2). In mice, there is evidence that the ability of mast cells to release large amounts of proteases (and perhaps heparin) permits these cells to enhance host resistance to the venoms of poisonous reptiles and arthropods and also to limit the toxicity of certain endogenous peptides, when exposure to such substances induces mast-cell degranulation and rapid release of the stored mediators[66–70] (Fig. 2). Some mast-cell proteases (such as mMCP-6) contribute to the ability of the cells to recruit neutrophils to sites of bacterial infection[64]. Indeed, enhancing host resistance to toxins and inducing acute inflammation in response to pathogens may represent phylogenetically ancient innate functions of this cell type.

In humans, mast cells have been categorized on the basis of their protease content into those that contain predominantly tryptase or (more rarely) chymase or both major proteases[71]

(Fig. 2). As chymase and tryptase have distinct substrate specificities[72,73], factors that can regulate the content of these two proteases in mast cells will in turn regulate the types of functions that the cells can serve after secretion of their stored mediators. Similarly, in mice, different mast-cell populations vary in their proteoglycan content, with some populations containing abundant heparin in their granules (connective tissue or serosal mast cells) or some having little or no heparin (mucosal mast cells)[52] (Fig. 2), and this will obviously influence the types of proteoglycan-dependent functions of each mast-cell subset.

However, *in vitro* studies show that populations of human mast cells that contain tryptase but little or no chymase and are maintained in SCF-containing media can have more chymase after incubation with IL-4 (refs. 74–77), IL-6 or IL-1β[77], TGF-β1 (ref. 78) or lipopolysaccharide[77] and that mouse mast cells lacking heparin can be induced to synthesize and store heparin after contact with fibroblasts[79] or exposure to SCF[52]. Although the extent to which such changes are reversible in individual cells is not clear, it has been shown that single mouse peritoneal mast cells that do contain heparin can give rise *in vitro* to clonal populations of mast cells that contain little or no heparin and that when such mast-cell populations that lack heparin are injected *in vivo* into mice genetically deficient in mast cells (WBB6F$_1$-*Kit*$^{W/W-v}$ mice), the cells become or give rise to cells that contain heparin[80].

Mast cells in adaptive immunity

Mast cells in mice can store a substantially larger number of different proteases than can those in humans[73,81], and the protease content of mast-cell populations in particular anatomical sites, such as the small intestine, can change during the course of infections associated with considerable increases in mast-cell numbers (such as infection with *Trichinella spiralis*)[81,82]. Because important mast-cell functions in such adaptive immune responses are thought to depend on the secretion of proteases from the cells, then factors that regulate this aspect of the cellular phenotype will in turn regulate mast-cell functions.

Mast-cell functions can also be altered via the types and amounts of receptors displayed by the cells, which renders the cells better able either to enhance or even (in some models) to suppress particular acquired immune responses. In T_H2-associated allergic disorders or responses to parasite infection, high concentrations of circulating IgE result in high surface expression of FcεRI by tissue mast cells (and circulating basophils), which in turn can enhance the IgE-dependent effector functions of such cells[83]. In mice, IgE- and antigen-dependent mast-cell activation can be markedly enhanced by exposure of the cells to interferon-γ[84], a cytokine that also enhances proinflammatory actions of macrophages (Fig. 1). In contrast, in certain models of severe contact-hypersensitivity responses, both *in vitro* and *in vivo* evidence suggests that the development of hapten-specific IgG1 antibodies during the response[85], combined with a change in the cytokine milieu at the site of the pathology, permits mast cells that had been adoptively transferred to that site to limit the extent of inflammation and pathology observed in WBB6F$_1$-*Kit*$^{W/W-v}$ mice or C57BL/6-*Kit*$^{W-sh/W-sh}$ mice genetically deficient in mast cells[85,86], at least in part by enhancing the ability of the mast cells to secrete IL-10 via an FcγRIII-dependent mechanism[85]. The evidence that stimulation of skin mast cells via immune complexes of IgG1 may enhance their secretion of IL-10 is reminiscent of a similar effect of immune complexes on tissue macrophages (Fig. 1).

However, evidence from work with mice with mast cell–specific inactivation of *Il10* has not confirmed a role for mast cell–derived IL-10 in the suppression of contact hypersensitivity[87]. Moreover, studies of mice rendered inducibly or constitutively deficient in mast cells independently of mutations affecting the gene encoding c-Kit have indicated, as did prior studies of IgE-deficient mice or mast cell–deficient WBB6F$_1$-*Kit*$^{W/W-v}$ mice[88],

that in the models of contact hypersensitivity tested, mast cells function mainly to enhance the response, including acting by means of effects on the sensitization phase[87]. Such findings suggest that the greater intensity of certain contact-hypersensitivity responses in WBB6F$_1$-*Kit*$^{W/W-v}$ or C57BL/6-*Kit*$^{W-sh/W-sh}$ mice may reflect abnormalities other than simply the mast cell deficiency of these mice (even though adoptive transfer of mast cells to such mice can limit the extent of their contact-hypersensitivity responses)[85,86].

In addition to producing IL-10, mast cells can produce an enormous spectrum of cytokines, chemokines and growth factors, as well as histamine and other autocoid mediators, many of which can participate in the transition from innate immunity to adaptive immunity[52,54,57,59,61]. Some of these mast cell–derived products may function in part to alter the phenotype and actions of other myeloid cells. For example, mast cells represent a potential source of TNF, which can influence the functions of macrophages and neutrophils, and of both IL-4 and IL-13, which can induce the M2 phenotype in macrophages (Fig. 1). Indeed, evidence from studies of C57BL/6-*Kit*$^{W-sh/W-sh}$ mice indicates that mast cells are key drivers of an M2 response in a mouse model of chronic allergic inflammation of the airways, in which mast cells are required both for the much higher lung concentrations of IL-13 and for the greater production by the lungs of several factors associated with an M2 macrophage response[84].

On the basis of evidence derived mainly from c-Kit-deficient mice, mast cell have also been linked to the promotion of peripheral tolerance to skin grafts[89] and to modulating host stromal and immune responses to tumors (notably, depending on the model system, with consequences that can favor either the tumor or the host[60,90]). It will be important to reassess the roles of mast cells in such models with mice deficient in mast cells independently of mutations that affect c-Kit.

Basic biology of neutrophils

Neutrophils have shorter lives than do macrophages and mast cells, and unlike those cells, neutrophils are released into the blood as mature or nearly mature cells devoid of proliferative potential (Table 1). The estimate of the time that neutrophils spend in circulation has been extended more than tenfold from 5–10 hours to 5.4 days (ref. 91). Properly understanding how the lifespan and distribution of neutrophils are regulated is important for many reasons, including the obvious point that these features of neutrophil biology provide the temporal and spatial context in which neutrophil phenotypic and functional variation can occur. Neutrophils are estimated to be produced in the bone marrow at a rate of roughly 1×10^9 cells per kilogram body weight per day during the steady state[92]. However, maintaining a concentration of 3×10^6 neutrophils per ml blood would require that neutrophils disappear from the blood after 5 hours and not after 5 days (ref. 91). Thus, either the rate of production of neutrophils or the validity of the assumptions made to estimate the duration of neutrophils in the circulation must be reconsidered[93]. The hallmarks of neutrophils—cytoplasmic granules—are produced sequentially during maturation in the bone marrow, which results in a heterogeneous population of granules ranging from the azurophil granules produced at the promyelocyte stage to the gelatinase granules produced at the metamyelocyte and band-cell stage. The rate of granule production and the time allocated to maturation, and thus to the loading of cargo into granules, may be influenced by cytokines and growth factors in response to infection and inflammation[30,94–99].

Regulation of the phenotype and function of neutrophils

Circulating neutrophils may be viewed as cells that have temporarily stopped their development, as they are not attached to any matrix and must remain functionally dormant so as not to obstruct the microcirculation[100]. Neutrophils become activated when caught by

activated endothelial cells at sites of inflammation and/or infection and are activated further during their passage into tissues, where they can begin a new round of transcription of genes encoding modulators of the inflammatory response, such as IL-8, MIP-1α (CCL3) GRO-β (CXCL2), VEGF and IL-1β[101], release their granules by exocytosis and mount a respiratory burst, which all contribute to the optimization of conditions for eradicating infecting microorganisms[102] (Fig. 3). Type I interferons generated during microbial infection[103] stimulate the recruitment of neutrophils and enhance phagocytosis via induction of the production of the chemokine CXCL10 (ref. 104).

The oxygen tension in inflamed tissues is diminished by edema and the consumption of oxygen by the NADPH oxidase activity of the phagocytes that accumulate, as NADPH oxidase transfers electrons from intracellular NADPH to molecular oxygen to produce the reactive free radical superoxide. Superoxide, which can be produced in phagosomes or extracellularly, can spontaneously form hydrogen peroxide that undergoes further reactions to generate other reactive oxygen species. Hypoxia stabilizes hypoxia-induced factors, among which the transcription factor HIF-1α is prominent in phagocytes[105]. The transcription factor NF-κB is an important activator of HIF-1α and vice versa, and activation of NF-κB is a key activator of the proinflammatory activities of macrophages, neutrophils, mast cells and endothelial cells. In neutrophils, activation of NF-κB results in both the activation of inducible nitric oxide synthase and hence the production of nitric oxide and the synthesis and release of proinflammatory cytokines such as IL-8, TNF, IL-6 and IL-1β[106]. At sites of infection, NF-κB activation can be induced in neutrophils by activators of TLRs, TNF or IL-1β[107]. In such settings, transcription of the gene encoding HIF-1α will consequently be great and stabilization of HIF-1α mRNA by hypoxia will result in downstream effects of this transcription factor, including enhanced production of antimicrobial proteins and inducible nitric oxide synthase. The myeloid transcription factor KLF2 has been shown to be a key inhibitor of NF-κB-dependent transcription of the gene encoding HIF-1α and thus acts as a tonic repressor of myeloid-cell activation[108].

The cytokine G-CSF is an important activator of neutrophils during bacterial infection, and one critical downstream target of G-CSF is CEACAM1 (CD66a), a transmembrane glycoprotein of specific granules and gelatinase granules of neutrophils[109]. Stimulation of neutrophils with G-CSF results in phosphorylation of the immunoreceptor tyrosine-based inhibitory motif of CEACAM1. Such phosphorylated CEACAM1 recruits the tyrosine phosphatase SHP-1, which acts as a bridge between CEACAM1 and the receptor for G-CSF and dephosphorylates that receptor, thus inhibiting its further signaling[110]. CEACAM-1-deficient mice not only have moderately more neutrophils but also a higher rate of death after challenge with *Listeria monocytogenes* due to the enhanced production of proinflammatory cytokines such as IL-1β and TNF by CEACAM-1-deficient neutrophils, which shows that enhanced stimulation of phagocytes can come at a price[110].

Neutrophils in innate immunity

It is apparent that multiple signals that may be present at sites of inflammation or infection, including cytokines and growth factors, can influence individual phenotypic features of neutrophils in the tissues, and if such signals reach the bone marrow, they may influence the phenotype of neutrophils during their production at that site. Such factors can regulate the maturation of neutrophils, as well as the local responses of the cells to inflammation (Fig. 3). However, neutrophils are relatively short-lived cells that lack proliferative potential and are destined to die in the tissues to which they are recruited, and it is not yet clear to what extent locally induced phenotypic changes in neutrophils are stable or fully reversible. Accordingly, in contrast to the definition of subsets of T cells, macrophages and mast cells,

there have been few attempts to define ‘subsets’ of neutrophils on the basis of their phenotype.

However, functionally important phenotypic features of neutrophils can vary stably from person to person. For example, human neutrophils can variably express CD177, a glycosylphosphatidylinositol-anchored protein located mainly on the membrane of specific granules and to the plasma membrane[111]. Its expression is influenced by polymorphisms of the gene encoding CD177 (ref. 112). CD177 is present in 0–100% of neutrophils, but the frequency of $CD177^+$ neutrophils seems to be constant in each person[113]. CD177 interacts with CD31 (PECAM-1), which is present on platelets and endothelial cells and anchors the anti-neutrophil cytoplasmic autoantibody (ANCA) antigen PR3 to the surface of the $CD177^+$ subset of neutrophils[114]. Only neutrophils that express CD177 are activated by ANCA to generate superoxide anions and to degranulate. The signal transduction depends on the association of CD177 with the integrin $\alpha_M\beta_2$ (Mac-1; CD11b) in lipid rafts and is mediated by Mac-1 (ref. 115). Therefore, it is possible that variation in the extent of neutrophil expression of CD177 among different people may influence the extent to which neutrophils can contribute to pathology in PR3-ANCA–associated small-vessel vasculitis[115].

Neutrophils in adaptive immunity

Although the innate and adaptive immune systems operate very efficiently to prevent and combat infections, their ability to combat cancers is dismal. Many publications have indicated that a subset of neutrophil-like myeloid cells is the main suppressor of T cells in tumor-bearing mice and in patients with cancer and thus inhibit the elimination of tumors by T cells. These are the aforementioned MDSCs[116]. In the mouse, MDSCs were initially characterized as having a $CD11b^+Gr\text{-}1^+$ phenotype, which encompasses both neutrophils and monocytes[117], but these can be distinguished by the expression of the marker Ly6G, which is present on neutrophils but not on monocytes, and by the expression of the marker Ly6C, which is high on monocytes but low on neutrophils[118]. It is now thought that there are two distinct MDSC subsets: granulocytic MDSCs have a $CD11b^+Ly6G^+Ly6C^{lo}$ phenotype, whereas MDSCs with monocytic morphology are $CD11b^+Ly6G^-Ly6C^{hi}$. GM-CSF and IL-6 are particularly potent inducers of MDCSs that do so by activating the myeloid transcription factor C/EBPβ[119]. Evidence indicates that $Ly6G^+$ granulocytic MDSCs have a major biological role in mouse models, and they can be detected in patients with cancer, but it is not clear that these cells constitute a particular developmentally regulated, stable subset of neutrophils. Instead, it seems more likely that such cells are neutrophils that are produced or phenotypically altered through the influence of cytokines and growth factors secreted by the tumors and/or by stromal cells reacting to the tumor cells. Such a hypothesis is consistent with the finding that cancers can induce a profile of myelopoiesis-stimulating growth factors and chemokines much the same as that seen during infection and inflammation[120,121]. Indeed, neutrophil MDSCs share many features with the neutrophils present in mice challenged by polymicrobial infection[122,123]. Tumors can produce many factors that can influence hematopoiesis, including IL-1β[96,124], CCL2 (refs. 118,125), TGF-β[126], G-CSF and GM-CSF[127], which of course can also be produced during bacterial infection. In line with that, the transcription factor C/EBPβ, which is critical for emergency granulopoiesis in response to infection[128], is also critical for the generation of MDSCs in both mice and humans[119].

Neutrophil MDSCs contain large amounts of arginase I (a protein located in gelatinase granules of human neutrophils). Arginase I is probably one of the main suppressors of T cells, as deficiency in L-arginine inhibits T cell proliferation and function[129] and arginase I converts L-arginine to L-ornithine, a precursor of polyamines and proline, which supports cell proliferation[130] and collagen synthesis[131] (Fig. 3). Neutrophil MDSCs also have

relatively high NADPH oxidase activity; this results in generation of hydrogen peroxide, which again inhibits T cell function[132]. Nitric oxide synthase, another consumer of L-arginine, is induced in MDSCs and inhibits T cell function[124,133]; depletion of cysteine and cystine by MDSCs also has been demonstrated to inhibit T cell function[134].

Concluding remarks

Having spent many years attempting to understand the biology and functions of the three myeloid cell types featured here, we are struck by how much there still is to learn. This is particularly true about the understanding of the regulation of macrophage, mast cell and neutrophil function *in vivo*; although many tools are available for investigating the functions of these cells in mice, the ability to assess the roles of these cells in humans is much more limited. Nevertheless, we have pointed out here that in both mice and humans, it is possible to identify 'subpopulations' of these cells that vary in phenotype and function. We therefore think that it is reasonable to conclude that regulating the development, stability and lifespan of such 'subpopulations' can represent a general strategy with which to ensure that macrophages, mast cells and neutrophils exert the appropriate functions during particular phases of innate and adaptive immune responses.

For both macrophages and mast cells, there is evidence (particularly from studies of mice) that cytokines and other microenvironmental factors present during an acquired immune response can alter the cells' role from mainly promoting inflammation at the onset of the response to limiting the magnitude or duration of the response once it has developed[31,85,86]. Like macrophages and mast cells, neutrophils can also contribute to the resolution of inflammation via multiple mechanisms[135,136]. One such mechanism may be to undergo a change in phenotype that alters the production of lipid mediators by neutrophils from those that promote inflammation (such as prostaglandins and leukotrienes, produced early in the inflammatory response) to those that can have anti-inflammatory effects (such as lipoxins)[137]. In addition, the ingestion of apoptotic neutrophils by macrophages can induce the macrophages to produce derivatives of omega-3-polyunsaturated fatty acids (resolvins and protectins) that contribute to the blockade of further recruitment of neutrophils and that have other effects that actively promote resolution of the inflammation[137]. The last example is just one of many potentially important interactions among the three cell types featured here, some of which may enhance and others that may help resolve inflammatory responses.

Although each of the myeloid cells discussed here can have variation in phenotypic features that may influence cellular functions, there may be substantial differences among these cell types in the stage in their development at which such changes can occur. For cells that retain proliferative potential (mast cells and perhaps macrophages), phenotypic alterations may occur both in individual post-mitotic cells and in the population as a whole as it undergoes expansion. In contrast, neutrophils do not proliferate, so their phenotypic plasticity must be regulated in the context of the relatively short lifespan of this terminally differentiated cell type.

Rather than attempting to subcategorize these myeloid cells into phenotypically distinct 'subpopulations' with different names, we think that it is more useful to consider that macrophages, mast cells and neutrophils each consist of populations of individual cells with a broad spectrum of phenotypes and functions and that some key characteristics of these cells are subject to substantial microenvironmental modulation, particularly in the dynamic contexts of innate or adaptive immune responses (Box 1). The regulation of cellular functions may occur through the effects of factors that influence the phenotype and function of the mature cells and also by factors that alter the differentiation and/or maturation of the cells. For example, for mast cells analyzed *in vitro*, incubation with particular cytokines,

such as SCF[138], IL-4 (ref. 139) or TGF-β1 (ref. 78), that can induce evidence of more mast-cell maturation can also alter the types or amounts of mediators released from the cells after aggregation of FcεRI (the main mechanism for eliciting antigen-specific mast-cell function). This type of relatively rapid regulation, via transient increases in the amount of growth factors or cytokines, may be particularly important during innate or adaptive immune responses in which there is rapid population expansion of macrophages, mast cells and/or neutrophils. Regulation of function can also occur via alteration of the progenitor populations that give rise to the mature lineages. For example, evidence indicates that sepsis can result in epigenetic changes in hematopoietic progenitor populations that in turn may be reflected in altered (in this case, diminished) function of their differentiated progeny[140]. This kind of regulation may be especially important for neutrophils, given their short lifespan and lack of proliferative ability.

Box 1

Mechanisms that can contribute to changes in myeloid cell phenotype and function

1. Epigenetic changes affecting progenitor cell populations (unless these also affect the hematopoietic stem cells, such changes may be transient as new progenitors are generated from hematopoietic stem cells)
2. Constitutive differences in anatomical microenvironments that induce local variation in the phenotype of the myeloid populations that develop at those sites; for example, the different types of macrophages, such as osteoclasts, Kuppfer cells, tissue macrophages, and so on (Fig. 1); connective tissue versus mucosal mast cells (in mice and rats); or mast cells containing tryptase versus mast cells containing tryptase and chymase (in humans; Fig. 2)
3. Factors that influence the maturation of the cells (for example, during population expansion in the context of inflammatory or immune responses) and factors that alter the cells' functional repertoire (for example, the acquisition by mast cells, during their maturation, of the ability to synthesize and store heparin or particular serine proteases)
4. Factors that alter phenotypic features of the mature populations (for example, transition between M1 and M2 macrophage populations, acquisition by neutrophils or macrophages of a myeloid suppressor cell phenotype in a tumor-bearing host, development by mast cells of a phenotype that permits enhanced production of IL-10)

Together such findings suggest that in addition to genetic factors, the microenvironment can regulate the phenotype and function of differentiated myeloid cells at the level of their progenitors, during their lineage-specific differentiation and after they have matured into the fully differentiated cell types. Although we have focused on macrophages, mast cells and neutrophils here, it is likely that this general principle also applies to other myeloid cells and to lymphocytes.

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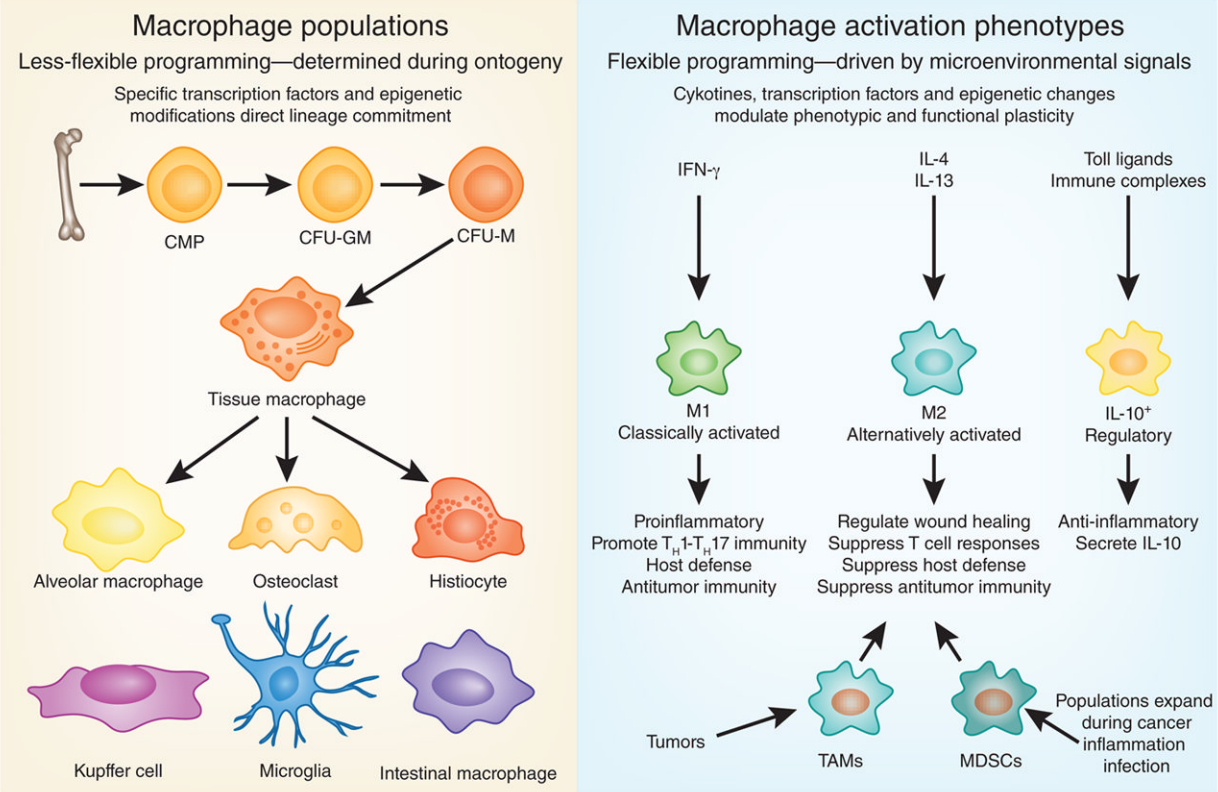

Figure 1.

Macrophage populations and functional subsets. Macrophages can be subcategorized into specific populations on the basis of their anatomical location (left) and functional phenotype (right). Tissue-resident macrophages include alveolar macrophages (lungs), histiocytes (interstitial connective tissue), osteoclasts (bone), microglia (brain), intestinal macrophages, Kupffer cells (liver) and so on. Mononuclear phagocyte subpopulations in the circulation can also differentiate into tissue macrophages after entering different anatomical sites; when activated by the appropriate stimuli, these cells differentiate into various subsets with distinct phenotypic and functional characteristics.

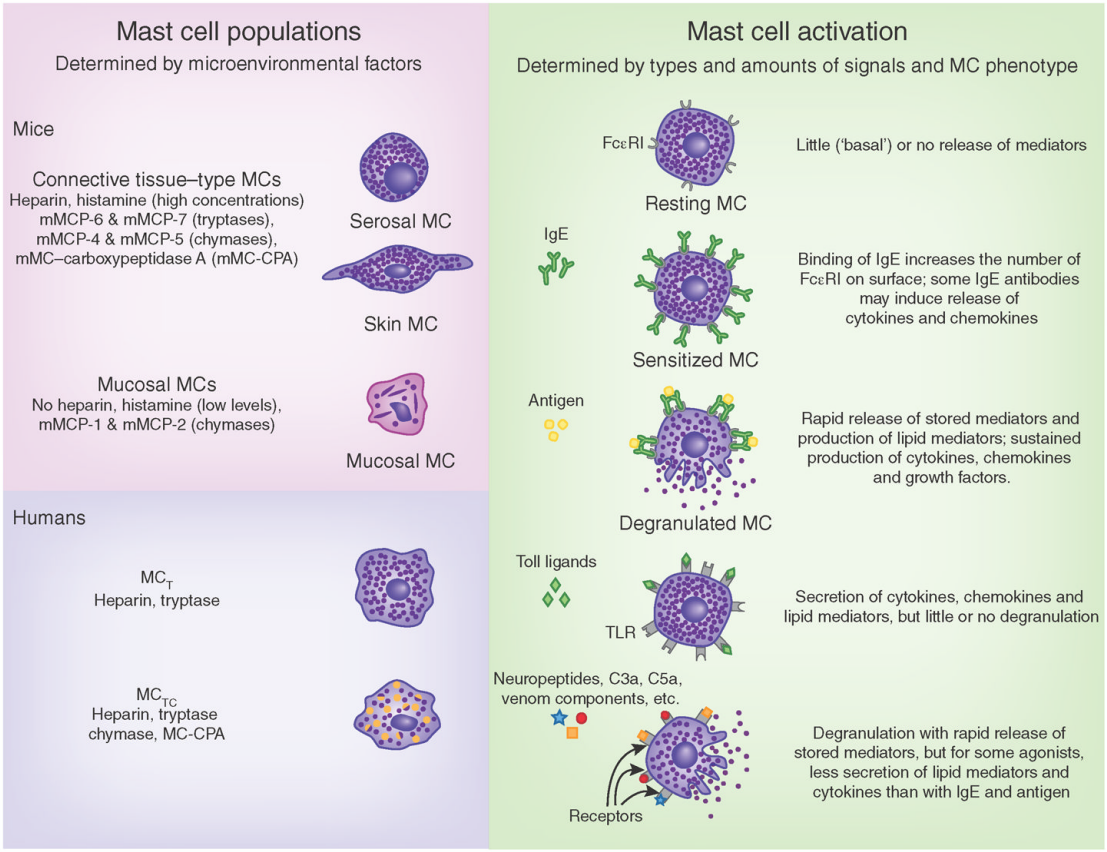

Figure 2.

Mast-cell populations and patterns of functional activation. Mast cells (MCs) in mice or humans can be subcategorized (left) into populations defined by anatomical location and/or mediator content (such as proteoglycans (heparin versus chondroitin sulfates) or proteases (tryptases, chymases or MC-CPA)). In IgE-associated immune responses to allergens or parasites (top right), the activation of mast cells via crosslinking of IgE bound to high-affinity receptors for IgE (FcεRI) on the cell surface by bi- or multivalent antigens results in rapid exocytosis of the cytoplasmic granules (degranulation) and the production of lipid mediators (such as leukotrienes and prostaglandins) and the more sustained secretion of many cytokines, chemokines and growth factors. Although many of these mediators have proinflammatory effects, others can have effects that suppress inflammation or promote tissue remodeling or repair. Signals not dependent on IgE (bottom right) can elicit different patterns of mediator release in mast-cell populations that express receptors appropriate for such ligands. Microenvironmental factors can influence the phenotype of mast cells that develop under basal conditions in different anatomic sites (left), including those phenotypic features that permit mast cells to respond to various ligands (such as the pattern of expression of receptors for those ligands) or to produce different mediators (right). TLRs are examples of the many pattern-recognition receptors expressed by various populations of mast cells. MC_T, mast cell containing mainly tryptase; MC_{TC}, mast cell containing both tryptase and chymase; C3a and C5a, anaphylatoxins of the complement system.

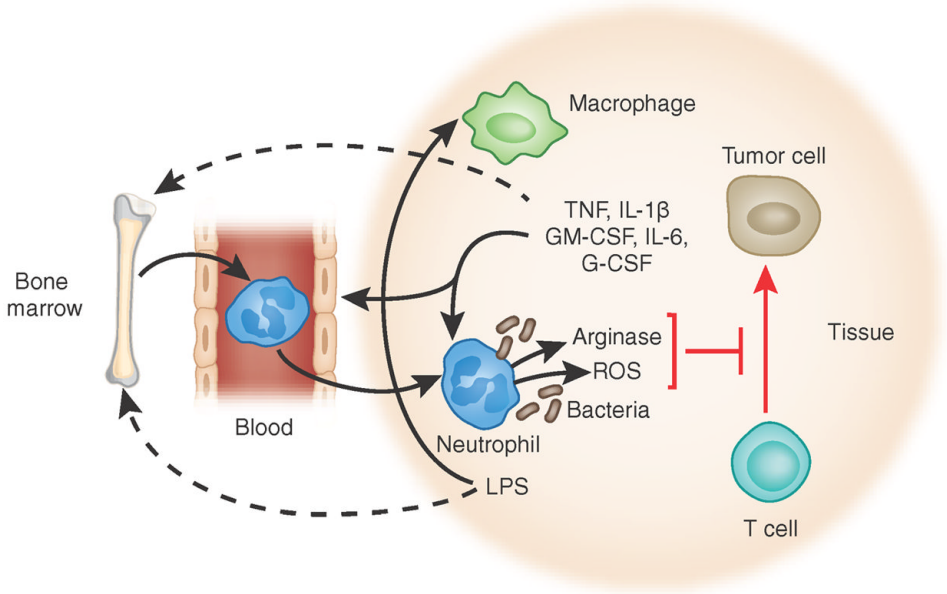

Figure 3.

Features shared by ‘neutrophil MDSCs’ and neutrophils in mice with polymicrobial infection. Growth factors and cytokines generated by tumors and macrophages, as well as bacterial products, modulate the development and phenotype of neutrophils by acting both on developing neutrophils in the bone marrow and locally on neutrophils in tissues. Mast cells (not shown here) also can generate many cytokines and growth factors that can influence neutrophils, including TNF, IL-1β, GM-CSF and IL-6. Reactive oxygen species (ROS) and arginase secreted from activated neutrophils can inhibit T cell function and permit tumor growth. In this setting, such neutrophils constitute ‘neutrophil MDSCs’. LPS, lipopolysaccharide.

Table 1

Natural history and main functions of macrophages, mast cells and neutrophils

Characteristic	Macrophages	Mast cells	Neutrophils
Difference in phenotype	F4/80$^+$ (mouse) or EMR1$^+$ (human), CD107b$^+$ (Mac-3$^+$), CD68$^+$	c-Kithi, FcεRIhi (including the $\alpha\beta\gamma\gamma$ form of FcεRI; some macrophages and neutrophils can express the $\alpha\gamma\gamma$ form of FcεRI); prominent cytoplasmic granules, some of which contain tryptase and/or contain heparin	Ly6G$^+$, MPO$^+$, polylobed nucleus
Origin of precursor cells	Bone marrow	Bone marrow	Bone marrow
Site of maturation	Almost all tissues (a few in the bone marrow)	Almost all tissues (a few in the bone marrow)	Bone marrow
Mature cells in the circulation	No (or very few)	No (except in mast cell disorders such a mastocytosis)	Yes
Mature cells recruited into tissues from circulation	No (immature monocytes migrate into tissues)	No (mast cell progenitors migrate into tissues)	Yes (during innate or acquired immune responses)
Mature cells normally reside in connective tissues	Yes	Yes	No (not detectable by microscopy)
Proliferative ability of mature cells	May vary by subpopulation (M2 macrophages can proliferate under certain circumstances)	Yes (under certain circumstances)	None reported
Lifespan	Weeks to months	Weeks to months (on the basis of studies of rodents)	Days (like other granulocytes)
Phenotypically distinct subpopulations in different tissues	Yes (Fig. 1)	Yes (Fig. 2)	Not reported
Phagocytosis	Yes	Reported, but biological importance not fully understood	Yes
Detect pathogens and danger signals and help to initiate inflammation	Yes (including near surfaces exposed to the environment)	Yes (including near surfaces exposed to the environment)	Yes (for example, in the blood or at sites of ongoing inflammation)
Enhance inflammation	Yes	Yes	Yes
Limit or suppress inflammation	Yes	Yes	Yes
Promote tissue repair	Yes	Yes	Yes
Antigen presentation	Yes	Reported, but biological importance is uncertain	Reported, but biological importance is uncertain
Degrade or detoxify components of animal venoms	Not reported	Yes	Not reported