Differentiation of human basophils: an overview of recent advances and pending questions

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Abstract: Basophils are rare, circulating leukocytes derived from hematopoietic CD34$^+$ progenitors. The identification of cytokines promoting their development in vitro has led to substantial advances in understanding their differentiation process. An important role could be assigned to interleukin-3 (IL-3), which supports the maturation of hematopoietic progenitors into basophils in vitro and in vivo. In contrast to other myeloid lineages, a specific basophil growth factor has not yet been discovered. Furthermore, it is still unclear whether basophils possess a lineage-restricted progenitor or whether they share a common ancestor with mast cells (MC), eosinophils, or even megakaryocytes. Partial answers to these questions could be provided using in vitro culture systems or taking advantage of hematological disorders, such as chronic and acute myeloid leukemia (CML and AML), some myelodysplastic syndromes, and the very rare acute basophilic leukemia in which basophilic differentiation occurs. *J. Leukoc. Biol.* 71: 557–564; 2002.

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INTRODUCTION

In 1879, Paul Ehrlich first described basophils. They represent the smallest fraction of white blood cells in healthy individuals (<1%). Their typical granular staining property, i.e., metachromasia, is shared by mast cells (MC), which have been compared or confounded frequently with basophils in spite of their distinct tissue localization. Indeed, mature basophils are present in the bloodstream, and MC leave the bone marrow as immature cells and undergo tissue-specific differentiation. Based on their similarity to MC, basophils have often been considered as minor and possibly redundant "blood MC." Like MC, basophils possess high-affinity immunoglobulin E (IgE) receptors (FcεRI) that are cross-linked upon engagement of receptor-bound IgE with corresponding antigens, resulting in the release of several mediators that are in part common for both cell types. Nevertheless, recent findings have provided new insights into the possible role of basophils in allergic disease and immunity to pathogens. Most notably, the discovery that basophils rapidly produce large amounts of the regulatory cytokines interleukin (IL)-4 [1] and IL-13 [2] has led to the idea that basophils might exhibit additional functions beyond their recognized role as effector cells in IgE-mediated reactions. Of note, several nonantigen-specific stimuli, derived from various organisms, have been shown to induce mediator or cytokine release from basophils. For example, protein Fv, an endogenous Ig-binding protein released in the intestine of patients affected by viral hepatitis, as well as the HIV-1 glycoprotein gp 120 have been shown to induce IL-4 and IL-13 production from basophils by binding to the VH 3 region of IgE [3, 4]. Similarly, soluble egg antigens derived from the parasite *Schistosoma mansoni* induce the release of IL-4 and other mediators from basophils of nonimmune donors [5]. Thus, because basophils rapidly release considerable amounts of IL-4 upon various stimulations, they may have a critical impact on the outcome of a primary infection by inducing T-cell differentiation to the T-helper cell type 2 (Th2) phenotype.

Like all blood leukocytes, the basophil derives from the common hematopoietic pluripotent stem cell in the bone marrow, where it differentiates completely before entering the bloodstream. It can stay there for an undetermined lapse of time, ready to migrate into various tissues during allergic and/or inflammatory events. In recent years, the understanding of the mechanisms governing the differentiation of basophils from hematopoietic stem cells has benefited greatly from the advances in cytokine research, allowing the growth-factor requirement to be defined in vitro. Nevertheless, the fact that basophils are the only myeloid cell population for which no specific growth factor has been identified to date has led some authors to propose that mature basophils are generated "by default" in the absence of growth factors.

Another important issue concerning basophilopoiesis is the identity of its lineage-restricted progenitor. Indeed, it remains unknown whether an ancestor with this unique differentiation potential exists or whether basophils derive from hybrid progenitors, which share MC, eosinophil, and/or megakaryocyte differentiation potential.

Thus, the purpose of the present overview is to summarize some of the recent advances in basophil research, focusing on the data that shed a new light on the differentiation of these

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cells. Before developing these data, we will describe the major phenotypic characteristics of these cells to facilitate the understanding of their relationship with the other hematopoietic elements.

PHENOTYPIC CHARACTERISTICS OF HUMAN NORMAL BASOPHILS

Morphology

After May-Grünwald-Giemsa staining of blood smears, basophils appear as mononucleated cells of 10–14 μm diameter, with a nucleus containing condensed chromatin. This nucleus, said in "clover" or in "brioche," contains lobes, separated by fissures, generally sealed at the extremities, resulting in a round or oval global appearance. The cytoplasm is filled with many round or oval, purple, dark granules of variable size, partially covering the nucleus (**Fig. 1A**). Basophilic granulocytes can also be generated in vitro in culture systems of human hematopoietic progenitors. In this case, they may present some slightly immature morphological features, as shown in Figure 1B. Electron microscope examination of mature circulating basophils showed that most granules are filled with dense particles of approximately 20 nm diameter. These granules appear homogeneous generally or exhibit myelinic figures. By contrast, when basophils are derived in vitro from human hematopoietic progenitors, their granules appear partially or totally extracted, as shown in Figure 1C. Their size is larger than that of MC, but they are less numerous. In addition to these specific granules, a smaller population of vesicles containing acidic phosphatases and corresponding to lysosomes can be distinguished.

Major antigens expressed by basophils

Mature basophils display a variety of surface molecules; the most representative among them are listed in **Table 1**. FcεRI, whose expression on circulating basophils is closely regulated by serum IgE levels, is most characteristic of this cell type [6]. Its aggregation by IgE-antigen complexes triggers a cascade of intracellular signals that lead to degranulation and synthesis of various mediators involved in the development and maintenance of allergic and inflammatory symptoms (reviewed in ref. [7]).

Basophils also express two subtypes of IgG receptors (FcγRIIA and B) [8]. Activation of FcγRIIA with IgG-antigen complexes leads to mediator release, whereas FcγRIIB transduces inhibitory signals because of the presence of an inhibition motive [immunoreceptor tyrosin-based inhibition motive (ITIM)] in the intracellular sequence. Thus, coaggregation of FcγIIRA and FcγIIRB results in an overall inhibition, because the ITIM motive blocks the activating signal delivered through the immunoreceptor tyrosin-based activation motive sequence of FcγIIRA [9]. Similar inhibition is observed when FcεRI and FcγIIRB are coaggregated [9]. Activation of basophils also occurs in response to complement components that bind to their corresponding receptors [10–13].

Basophils display a number of growth-factor receptors (R), such as IL-2R, IL-3R, IL-4R, IL-5R, granulocyte-macrophage colony-stimulating factor (GM-CSF)R, nerve growth factor (NGF)R, and IL-8R [14, 15]. Contrasting with MC, c-kit (CD117) is displayed only feebly on basophils [16].

It is interesting that basophils express different types of chemokine receptors such as CCR1, CCR2, CCR3, CCR5, CXCR1, CXCR2, CXCR4, and CRTH2 [17–21]. Among these, CCR3 appears to be the most important, as judged by its high expression and because eotaxin induces a strong migration of basophils [19]. Although it remains to be proven, it is likely that the presence of CCR3 and other CC chemokine receptors shared between eosinophils and basophils account for the previously demonstrated, strong, temporal association of their influx into tissues, such as the nose [22] or the lung [23], during

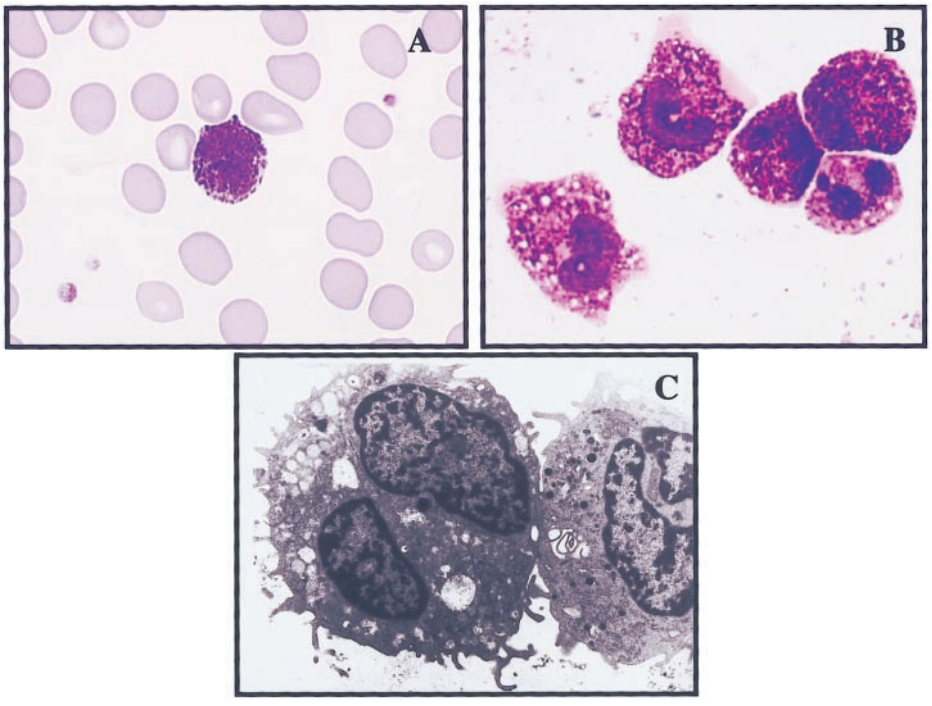

Fig. 1. Morphological appearance of human basophils. (A) May-Grünwald-Giemsa staining of a basophil in normal peripheral blood (original magnification, ×500); (B) May-Grünwald-Giemsa staining of several human basophilic cells obtained by long-term culture of $CD34^+$ cells from normal cord blood in the presence of IL-3 and TGF-β (original magnification, ×1000); (C) transmission-electron microscopy of human basophilic cells obtained by long-term culture of $CD34^+$ cells from normal cord blood in the presence of IL-3 and TGF-β (original magnification, ×6000).

TABLE 1. A Selection of Important Ags Expressed on Normal Human Basophils

CD or nonclustered Ags	Natural ligand
Complement receptors	
CD11b (Mac-1)	CD54, iC3b
CD11c (CR4; p150, 95)	C3bi, C3d, g, fibrinogen
CD21 (CR2)	C3d, g, EBV
CD35 (CR1)	C3b, C4b
CD46 (MCP)	C3b, C4b, measles virus
CD55 (DAF)	C4b/2a, C3b/Bb
CD59 (protectin, Mac inhibitor)	C5b-8, C5b-9, CD2
CD88 (C5aR)	C5a
Immunoglobulin receptors	
CDw32 (FcγRIIA and B)	IgG Fc (low affinity)
FcεRI	IgE Fc (high affinity)
Homing receptors and related molecules	
Selectins	
CD15s (Lewis-x, Lex)	P-selectin, E-selectin
CD62L (LAM-1, L-selectin, LECAM-1)	MadCam-1, GlyCAM-1, CD34
CD162 (P-selectin glycoprotein ligand-1, PSGL-1)	P-selectin, E-selectin
Integrins	
CD11a (LFA-1)	ICAM-1 (CD54), ICAM-2, ICAM-3
CD18	ICAM-1 (CD54), ICAM-2, ICAM-3
CD29	Collagen, laminin
CD44 (hermes antigen, Pgp-1)	Hyaluronic acid
CD49a (VLA-1)	Collagen, laminin
CD49d (VLA-4)	Fibronectin, VCAM-1
Receptors for cytokines	
CD25 (Tac; IL-2Rβ)	IL-2
CD116 (GM-CSFRa)	GM-CSF
CD123 (IL-3R)	IL-3
CD124 (IL-4R)	IL-4
CD125 (IL-5R)	IL-5
CD128 (IL-8R)	IL-8
IL-18R	IL-18
Receptors for chemokines	
CCR1	MIP-1α
CCR2	MCP-1
CCR3	Eotaxin
CD195 (CCR5)	CCR5
CXCR1	IL-8
CXCR2	GRO-α
CD170 (CXCR4)	SDF-1
CRTH2	PGD2
Other surface membrane structures	
CD40	CD40L
CD40L	CD40
CD13 (aminopeptidase N)	NA
CD203c (97A6, ectonucleotide pyrophosphatase/phosphoiesterase 3, E-NPP3)	NA
Bsp-1 (basophil-specific protein 1)	UN
212H6	UN

NA, nonapplicable; UN, unknown.

allergic late-phase reactions (LPRs). The actions of hematopoietic cytokines such as IL-3, IL-5, and GM-CSF offer more insight into tissue influx of basophils and their subsequent activation. Indeed, expression of these Th2 lymphocyte-derived cytokines was shown to increase in allergen-induced, cutaneous LPRs in atopics [24], and these cytokines have also been shown to facilitate basophil migration [25].

Basophils also display a number of adhesion molecules [26] and myeloid markers, such as CD13, CD26, CD45, CD33, CD43, CD44, CD54, CD11b, CDW17, CD31, and CD35 [27]. Typical myelomonocytic antigens, such as CD14, CD15, and CD16; natural killer (NK) cell markers (CD56, CD57); or B- and T-cell antigens (CD19, CD20, CD21, CD3, CD4, and CD8, respectively), are not detected on normal basophils [27–29]. Finally, basophils express ceramide monohexoside class I molecules [27] and CD63, which is displayed on the surface of granules in resting cells but becomes detectable on the cell membrane after activation [30].

Currently, there are four basophil-specific monoclonal antibodies (mAbs) that do not react with other circulating leukocytes or with MC: Bsp-1, 2D7, BB-1, and 212H6. Bsp-1, an IgM class mAb that recognizes an epitope expressed on the surface of human basophils, was originally raised against the human erythroblastic leukemia cell line (HEL) [31]. It reacts with a 45-kD surface antigen on HEL cells in Western blots [31].

2D7, an IgG1 mAb, reacts with a ligand localized to the secretory granules of human basophils [32]. Immunologic activation of basophils leads to the loss or attenuation of the staining intensity, indicating release (or decay) of the 2D7 antigen [32]. 2D7 mAb has been used successfully for immunohistochemical staining of basophils in human skin during the LPR after cutaneous allergen challenge [33].

BB-1 is an IgG2a mAb that recognizes an antigen localized mainly in the secretory granules, with a comparatively small expression on the cell surface [34, 35]. The BB-1 antigen is released upon activation with anti-IgE or the calcium ionophore A23187 [34]. Because the BB-1 antigen was not detected in any other cell type, the authors suggested its use as a discriminating marker of basophil activation, particularly in tissues where it has been used successfully for immunohistochemical detection of basophils [34].

Very recently, 212H6, an IgM mAb that recognizes an epitope expressed on the surface of human resting basophils, was obtained by immunization against purified $CD3^-$, $CD4^+$, and $CD11c^-$ tonsillar dendritic-cell (DC) precursors (unpublished results). With regard to leukocytes, 212H6 mAb stained only circulating human basophils and has been used to purify this cell subset to homogeneity. Apart from circulating basophils, 212H6 also stained epithelial cells in tonsils as well as keratinocytes in the skin but not $CD34^+$ cells or monocyte-derived DC (unpublished results).

ONTOGENY AND DIFFERENTIATION OF BASOPHILS

Although the phenotype of mature basophils has been studied extensively, the early stages of basophil maturation and their relationship to other cell lineages are not well understood. Basophils, as well as MC, monocytes, eosinophils, and neutrophils, are thought to arise from $CD34^+$ progenitors found in cord blood, peripheral blood, and the bone marrow [36]. In semi-solid medium, these progenitors differentiate into meta-

chromatic cells whose morphological, cytochemical, and immunophenotypical features are typical of basophils (reviewed in ref. [37]). Yet, the precise ancestor of the basophilic lineage remains controversial, because it has been described indifferently as a multipotent precursor [colony-forming unit (CFU-Mix)], as a bipotent eosinophil/basophil (CFU-Eo/Baso) or megakaryocyte/basophil (CFU-Mega/Baso) progenitor, or as a unipotent precursor (CFU-Baso) (reviewed in ref. [38]). To resolve this controversy, four experimental approaches have been used: development of liquid cultures ensuring the differentiation of basophils in the presence of well-defined cytokines; analysis of leukemic cell lines with basophil-differentiation potential; evaluation of histidine decarboxylase (HDC) activity in hematopoietic progenitors as an indicator of their basophilic potential; and phenotypic analysis using antibodies more or less specific for the basophilic lineage.

Cytokines affecting basophilopoiesis

IL-3

It is generally acknowledged that the main known growth and differentiation factor for basophils is IL-3, whereas the growth and development of MC require the presence of stem cell factor (SCF). In conformity with their relative cytokine requirements, mature basophils can be distinguished phenotypically from MC by their differential surface expression of the IL-3 receptor (CD123), which is displayed on basophils and other cells but not on MC, and the SCF receptor (c-kit/CD117), strongly expressed on MC but not, or only weakly, on mature basophils [14, 15].

Initially, IL-3 was termed histamine-producing cell-stimulating factor (HCSF) and was characterized as a T-cell-derived factor capable of inducing HDC synthesis and increased histamine production in murine hematopoietic progenitors [39]. The association of IL-3-induced histamine synthesis with the basophilic lineage has been demonstrated in rodents and in humans [40, 41]. Injection of IL-3 induces a consistent blood basophilia in primates, accompanied by a dose-dependent increase of the histamine content in circulating cells [42]. Therefore, it seems that in humans, IL-3 acts as a basophilopoietin deprived of a notable influence on MC differentiation, and its effect on the mucosal MC differentiation is preponderant in mice. However, this distinction between the two species is not as clear-cut, because IL-3 can increase histamine synthesis in rodent-basophilic precursors [43]; it is critical for the differentiation of rodent basophils in the course of the parasitic infestations [14]; it supports the development of FcεRI+ c-kit+ MC-like cells in long-term culture of human CD34+ cells in the presence of SCF [44]; and IL-3 facilitates the early phase of human MC differentiation by enhancing the expansion of hematopoietic progenitors in conjunction with SCF and IL-6 [45].

GM-CSF

GM-CSF shares with IL-3 the capacity to increase the production of histamine by rodent-hematopoietic precursors, although its efficiency is lower [46]. Additionally, GM-CSF stimulates the production of histamine in bone marrow cells of rhesus monkeys in liquid cultures [47]. This observation is consistent with its positive effect on the proliferation of bipotent basophil/eosinophil progenitors and with its capacity to induce the basophilic differentiation of the HL60 cell line in alkaline culture conditions [48]. Finally, a slight basophilia, as well as an increase of histamine content in circulating cells, occurs after injection of GM-CSF into monkeys [49].

Other cytokines

IL-5 is involved in the differentiation of eosinophils and basophils, because it allows the growth of colonies comprising basophils from peripheral stem cells [50] and facilitates the basophilic differentiation of hematopoietic lineages [50]. In this respect, it resembles NGF that acts in synergy with GM-CSF to favor the development of basophils [48]. Furthermore, transforming growth factor-β (TGF-β) seems to exert a positive effect on basophilic differentiation from human progenitors in the presence of IL-3, which probably results from inhibition of eosinophil differentiation rather than stimulation of basophilopoiesis [51]. Apparently, other cytokines, such as IL-1, IL-2, IL-4, IL-6, IL-7, IL-8, inteferons (IFNs), or tumor necrosis factors (TNFs), do not affect the development of this lineage, even if mature basophils express receptors for some of these molecules (Table 1) [15]. It should be kept in mind that the response of human leukemic cell lines to cytokines may differ from their normal counterparts. This is exemplified by the human immature basophilic cell line KU812, originally established from a patient with chronic myelogenous leukemia (CML) [52], which differentiates into basophil-like cells in response to several cytokines, including TNF-α and IL-6 [53] or IL-4 [54]. As shown by Hara et al. [54], incubation of KU812 cells with IL-4 resulted in several changes, namely a tenfold increase of total histamine content, appearance of metachromatic granules, and up-regulation of functionally active FcεRI within 7 days, concomitant with increased transcription of FcεRI α, β, and γ chain mRNA. Finally, it has been demonstrated recently that IL-18 acts in synergy with IL-3 to increase histamine and IL-4 production by mature basophils [55], raising the question of the role of IL-18 during basophil development.

Basophilopoiesis, a "default" differentiation pathway?

With the exception of basophils, all hematopoietic lineages depend on a specific factor for their terminal differentiation, such as erythropoietin for erythroid progenitors, G-CSF for neutrophils, IL-5 for eosinophils, M-CSF for monocytes, and thrombopoietin for megakaryocytes. The lack of one of these molecules in genetically modified mice results in a profound deficit of mature cells of the corresponding lineage. By contrast, basophil counts remain normal in IL-3-deficient mice [56]. In addition, IL-3 injection not only induces basophilia but also promotes various changes in other leukocyte populations in humans [57]. These two data are not compatible with a specific basophilopoietin function of IL-3, in spite of its capacity to stimulate basophil differentiation from hematopoietic progenitors in vitro [58, 59]. A possible explanation for this discrepancy might be that the final maturation steps occur "by default," without growth factor. In favor of this hypothesis, it appears that IL-3 is not required continuously in vitro to generate human basophils, because a 3–4 h exposure of cord-

blood progenitors to IL-3 is sufficient to drive basophilic differentiation during a subsequent 3-week culture period [60]. The cells generated by this procedure resemble circulating basophils even more than those generated in the constant presence of IL-3. Indeed, they are mostly $2D7^+$ $Fc\varepsilon RI^+$, express a series of integrins also found on normal peripheral blood basophils, display basophil-like morphology by light or electron microscopy, and release histamine after activation through FcεRI [60]. However, one cannot exclude the participation of factors produced endogenously or present in fetal calf serum in such a model.

HDC activity as an indicator of basophilic potentiality

The presence of high intracellular histamine levels has often been considered a criterion for basophil or MC differentiation. However, we have demonstrated that rodent and human hematopoietic progenitors can synthesize and release histamine prior to the formation of granules where it can be stored [41, 61–63]. IL-3 and GM-CSF, which exert a positive effect on basophil differentiation, increase histamine production strongly by inducing HDC activation [61]. This is exemplified in **Figure 2**, showing the early increase of HDC activity in human $CD34^+$ progenitors during basophil differentiation promoted by IL-3, as compared with the late HDC activation in progenitors cultured in a cocktail of cytokines driving MC differentiation. In the mouse, IL-3 induced an early and transient expression of HDC in a cell population committed to the basophil lineage but a more delayed and sustained expression of this enzyme in hematopoietic progenitors undergoing differentiation into MC [43]. Furthermore, recent studies conducted on human hematopoietic cell lines confirm that the HDC activity is associated with early events of basophilopoiesis and not mastopoiesis and might be taken as an indicator of inherent basophil potentiality [64], similar to the expression of glycophorine A or gpIIbIIIa, which reveals erythroid or megakaryocyte commitment, respectively.

Identity of basophil precursors

Basophils derive from $CD34^+$ stem cells that are most frequent in the bone marrow in adults. In vitro clonogenic assays have revealed that basophil and eosinophil lineages are often associated in the same colony, in agreement with the fact that major probasophilic cytokines also favor the growth of eosinophils in vitro (reviewed above). In these cultures, mixed basophil/eosinophil colonies are frequent, suggesting the existence of a bipotential precursor, CFU-Eo/Baso [50]. The association between basophils and eosinophils is also observed in vivo in humans, because the injection of IL-3 or GM-CSF induces basophilia and eosinophilia, as well as an increased number of precursors of both lineages [57]. The occurrence of granulocytes displaying a hybrid eosinophil/basophil phenotype in patients with CML or acute myelogenous leukemia [65] provides an additional argument for a common eosinophil/basophil progenitor.

Recent studies on human leukemic cell lines have led to the hypothesis that a common basophil/megakaryocyte precursor (CFU-Baso/Mega) might exist. Indeed, most megakaryocytic cell lines (UT7 and its subclones D1, HEL, CMK, LAMA84, and MTT95) express mRNA for HDC and synthesize histamine [64]. This biological activity is associated with the expression of several basophilic markers, such as the three chains of FcεRI and CCR3 and the capacity to synthesize IL-4 and IL-13 [64]. The existence of a common basophilic/megakaryocytic precursor is also in agreement with the fact that antibodies recognizing basophils are produced upon immunization with megakaryocytic cell lines. Bsp-1 and 97A6 antibodies, for instance, have been generated by immunizing mice against HEL and UT7 cells, respectively [31, 66].

Finally, it has been demonstrated that in certain conditions, the cell line UT7, whose megakaryocytic potential is clearly established, can exhibit features of basophilic as well as of eosinophilic differentiation, thus raising the question of a common baso/eosino/mega progenitor [67].

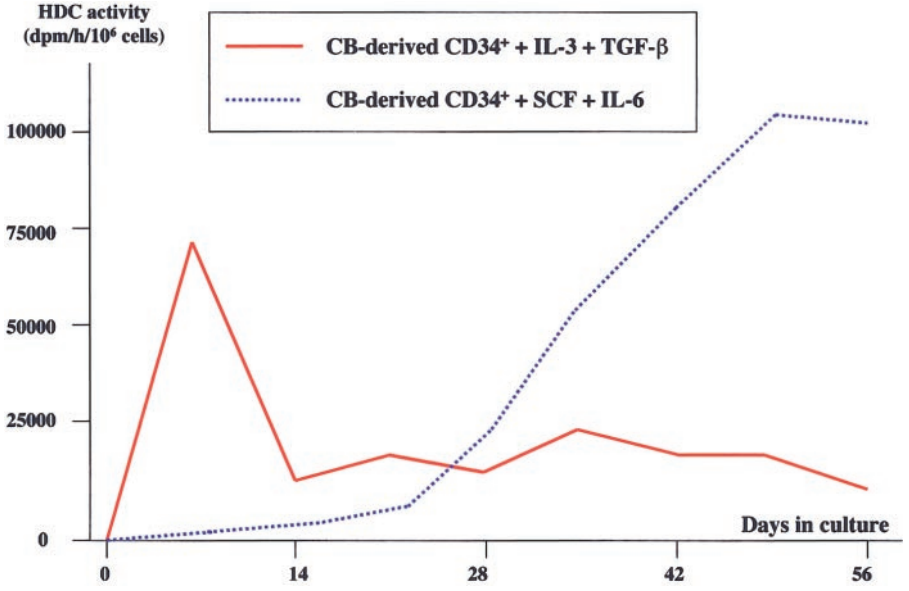

Fig. 2. Kinetics of HDC activity in human $CD34^+$ cells purified from cord-blood mononuclear cells in long-term cultures supporting basophil or MC differentiation. Cells were cultured in conditions favoring basophilic differentiation [human recombinant (hr)IL-3 at 5 ng/ml and hrTGF-β at 1 ng/ml] or MC differentiation (hrSCF at 100 ng/ml and hrIL-6 at 80 ng/ml). Once a week, cell aliquots were recovered, and HDC activity was determined in lysates by means of a radiochromatographic assay measuring the conversion of L-[^{3}H]histidine into [^{3}H]histamine. Data are expressed in dpm/h/10^6 cells. The maximum of differentiated cells (90–98%) was obtained within 3–4 weeks of culture for basophils and 6–8 weeks for mast cells.

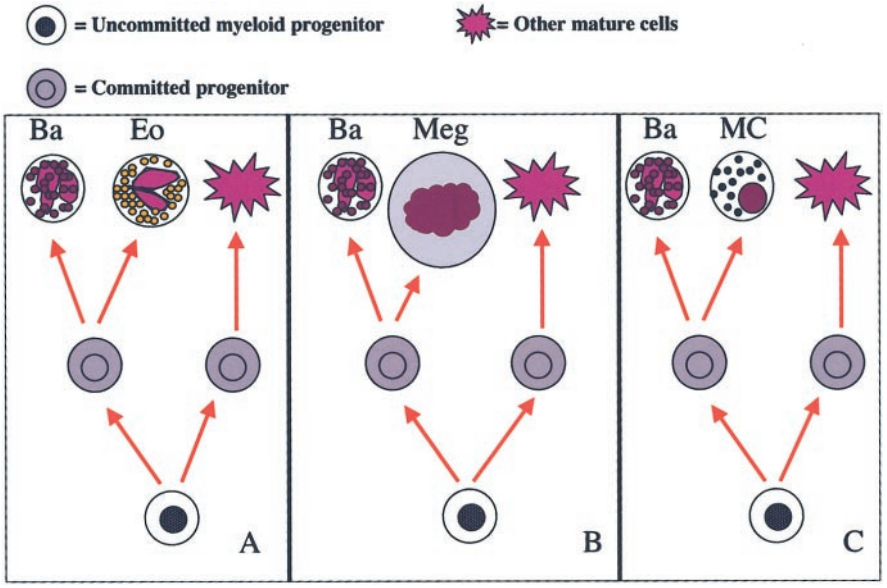

Fig. 3. Three possible pathways of basophil differentiation from uncommitted myeloid progenitors. (A) Hybrid progenitor with basophil/eosinophil-differentiation potential, supported by clonogenic assays in vitro. (B) Common ancestor shared by basophils and megakaryocytes, suggested by studies on leukemic cell lines. (C) Basophil/MC progenitor, supported by the description of cells sharing phenotypic features of both lineages in human pathology. Ba, basophil; Eo, eosinophil; Meg, megakaryocyte.

Differentiation of basophils and mast cells: a matter of controversy?

Basophils and MC share several morphological, biochemical, and immunophenotypic characteristics. Nevertheless, a common origin for basophils and MC is still a matter of debate. Recent studies tend to prove that these cells possess different precursors, basophils originating from cells that also generate eosinophils [68], and MC deriving from a primitive myeloid progenitor shared with monocytes and macrophages [45]. Indeed, human $CD34^+$ c-kit^+ $CD13^+$ cells give rise to MC and monocytes when cultured with SCF and IL-6, and they fail to generate basophils or other hematopoietic lineages [45]. Clinical data are also in favor of a dichotomy between basophils and MC precursors. For instance, no modifications of the basophil compartment have been described in patients suffering from mastocytosis [69]. Conversely, blastic transformation of CML is accompanied by an important increase in the number of basophils, and no modification of the MC compartment has been noted [70].

Although MC progenitors have been characterized as a separate lineage displaying a $CD34^+$ c-kit^+ $CD13^+$ phenotype, the description of a new mAb (97A6, clustered into CD203c) specific for mature MC and basophils and their progenitors has challenged this notion recently [66]. mAb 97A6 did not react with any other hematopoietic cell type [66] and therefore, may recognize an epitope associated with MC and basophilic commitment of a $CD34^+$ precursor distinct from that of other lineages. Yet, it should be noted that all basophilic progenitors belong to the $CD34^+$ $97A6^+$ cell population, and those giving rise to MC are also present among $CD34^+$ $97A6^-$ cells [66]. This could be explained by the heterogeneity of the MC population, provided that it is admitted that two distinct precursors can generate two types of mature MC effectively.

The possibility of a common origin of the MC and basophil lineage is also supported by the surprising observation that basophils with mast cell-like phenotypic features can be found in patients with asthma, allergy, or allergic-drug reactions [71]. These cells have a multilobulated nucleus and are $Bsp1^+$ but also express substantial amounts of the specific MC proteases (tryptase, chymase, and carboxypeptidase A), as well as c-kit [71].

The expression of mast cell markers c-kit and tryptase by basophilic KU812 cells [72, 73] and the significant percentage of cells displaying the basophilic marker Bsp-1 among the human mast cell line HMC-1 (unpublished results) provide further support for the existence of hybrid MC/basophil progenitors. Finally, it has been demonstrated recently that a significant proportion of neoplastic basophils from patients with CML, idiopathic myelofibrosis, or myelodysplastic syndrome contained tryptase in their cytoplasmic granules and expressed α-tryptase mRNA [74]. Therefore, the currently predominant concept that MC and basophils originate from separate lineages may have to be revised. The different hypotheses concerning the nature of the hematopoietic progenitor giving rise to basophils, supported by in vitro models or observations in vivo, are presented in **Figure 3**.

CONCLUDING REMARKS

New molecular and cellular approaches are still necessary to increase the present knowledge of the regulatory mechanisms governing the differentiation of the basophilic lineage. Indeed, the precise nature of the hematopoietic progenitor giving rise to the basophil lineage remains a matter of debate. In addition, the capacity of this progenitor to differentiate exclusively into basophils or basophils and MC, as suggested by the existence of hybrid c-kit^+ Bsp-1^+ $tryptase^+$ cells, has to be established. Furthermore, the cytokine(s) endowed with a biological activity restricted to basophil growth and differentiation has yet to be discovered. Nevertheless, our present understanding concerning the phenotype and differentiation pathway of these cells has already proven its usefulness in the follow-up of chronic inflammatory or allergic diseases, as well as some malignant disorders. Further investigation of the mechanisms governing the differentiation of these elements will also provide us with a better understanding of the biology of the cytokines, particu-

larly regarding their autocrine, paracrine, and endocrine properties.

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