REVIEW ARTICLE

Basophils: new players in the cytokine network

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ABSTRACT. Basophils belong to a myeloid cell population that has been ignored for more than a century, mainly because of its paucity, its lack of specific markers, and the absence of experimental models. Given that in mice, even the mere existence of basophils was contested, they were alluded to as "histamine-producing cells" or "non-T non-B cells" in initial studies. It is now widely acknowledged that basophils respond to various IgE-dependent or -independent stimuli, and are engaged in a complex cross talk with a number of immunocompetent cells (T or B lymphocytes, macrophages, dendritic cells, endothelial cells…). Indeed, on the one hand they are critically involved during the onset, the effector phase and exacerbation of T_H2 responses through their capacity to generate large amounts of cytokines with pro-T_H2 functions (IL-4, IL-13 TSLP, IL-25), on the other hand, they contribute to immunoglobulin synthesis and class switching, angiogenesis, autoimmunity, tumor immunity and hematopoiesis by producing cytokines such as IL-6, VEGF, GM-CSF and IL-3. Finally, it has been established that they can present antigens to $CD4^+$ or $CD8^+$ T cells in an MHC class II- or class I-dependent manner, respectively. Taken together, these activities confer important immunoregulatory functions upon basophils, both in innate and adaptive immunity.

Keywords: basophils, histamine, IL-4, immunoregulation, TH2 responses, allergy

Although Paul Ehrlich identified basophils only two years after mast cells in 1879 [1], this cell population was then neglected or ignored for more than a century, and was often mistaken as a circulating form of mast cells. Indeed, basophils are the least common granulocytes in the circulation, where they usually represent less than 1% of the white blood cell population. Furthermore, the absence of specific surface markers, at least in mice, has been a drawback to their purification. Progress in basophil research has also been hampered by apparent morphological and functional redundancies with mast cells and the lack of suitable murine experimental models, which explains why immunologists have taken little interest in this field. Indeed, although basophils are found in most vertebrates (mammals, birds, reptiles and amphibians), as well as in fish [2], with some variations as to their number and morphological features, in mice even their very existence was doubted until quite recently. This erroneous notion originated from the differences between murine and human basophil morphology, the former being markedly less granulated than the latter. Human basophils have only been recognized as the unique source of histamine among blood leukocytes since 1955 [3], while histamine synthesis in their murine counterpart was first reported in 1981, in response to a cytokine initially termed histamine-producing cell stimulating factor (HCSF) [4] that was later identified as IL-3 [5]. The corresponding biological activity, called HCSA (histamine-producing cell stimulating activity) [6, 7], was initially described in a model of allograft rejection, but was demonstrated later during anti-parasitic or mitogenic responses [4, 8, 9]. It resulted from a small subset of bone marrow cells originally named histamine-producing cells that have since been characterized as basophils [10].

Immunologists came to recognize basophils as potential immunoregulatory cells only when it was discovered that they represent one of most potent sources of IL-4 [10-14] and that they can migrate into lymph nodes to drive T_H2 polarization [15]. This notion was supported in 2009, 130 years after their discovery, by three distinct research groups who also demonstrated that, along with their capacity to produce IL-4, murine basophils could present antigens to naive T cells, thus promoting T_H2 cell differentiation during allergic and anti-parasitic immune responses [16-18]. These exciting data explain the recent rise in the number of publications dealing with basophils multiplied by four in 10 years (285, 305 and 1,294 publications in 1990, 2000 and 2009, respectively), 16% of the results being derived from murine models in 2009, as compared to 5% in 1990. The reappraisal of this rare cell population has recently prompted the search for its pharmacological modulation by inhibitors or regulators with potential clinical applications. The purpose of this review is to summarize the recent advances in this field of research, in terms of ontogeny, relationship with the mast cell lineage, phenotypical and functional characteristics as a source and a target of cytokines and, finally, means of regulation.

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BASOPHIL DIFFERENTIATION AND THEIR RELATIONSHIP WITH MAST CELLS

Like all other blood leukocytes, basophils and mast cells, derive from the common hematopoietic pluripotent stem cell in the bone marrow. They complete their maturation in the bone marrow before entering the bloodstream, conversely to mast cells that leave this site at a precursor stage to differentiate in peripheral tissues [19]. 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, thrombopoietin for megakaryocytes and SCF (c-kit ligand) for mast cells. The lack of any one of these molecules in genetically modified mice results in a profound deficit in mature cells of the corresponding lineage. Up to now, basophil-deficient mice have not been reported. Indeed, basophil counts remain essentially at a steady state level in mice that lack IL-3 [20], in spite of the fact that this growth factor promotes their *in vitro* differentiation from normal bone marrow cells [21, 22], mediates *in vivo* basophilia after injection [23], maintains their survival through a Pim1-dependent mechanism [24], and is required for their expansion during parasitosis [20]. Consequently, IL-3 does not qualify as a specific basophilopoietin. It has been proposed that the final maturation steps occur "by default," in the absence of a growth factor [19]. In favor of this hypothesis, it has been observed that IL-3 is not required continuously *in vitro* to generate human basophils, a 3-4 h exposure of cord-blood progenitors to IL-3 being sufficient to drive their differentiation during a subsequent three-week culture period [25]. The cells thus generated resemble circulating basophils even more than those obtained in the constant presence of IL-3, inasmuch as they express a series of markers also present on normal peripheral blood basophils, display basophil-like morphology assessed by light or electron microscopy, and release histamine after FcεRI crosslinking. However, the participation of factors produced endogenously or present in fetal calf serum cannot be excluded in these conditions.

The branching point between basophil/mast cell and lymphoid lineages seems to occur quite early in the differentiation scheme initiated from the hematopoietic stem cell. Based on colony-forming assays or phenotypical characteristics, bipotent basophil/eosinophil and basophil/mast cell progenitors have been described. Moreover, histamine can be produced and basophil markers expressed in erythroid/megakaryocytic cell lines, such as UT7, HEL and LAMA84 [26, 27], even though the physiological relevance of this finding remains to be established. Alternatively, some data suggest a relatively early separation between basophil and megakaryocyte/erythroid progenitors [28-30] *(figure 1)*. Starting from granulocyte/monocyte progenitors (GMP), the commitment towards basophil, mast cell and eosinophil lineages takes place under the control of two main transcription factors: the CCAAT enhancer-binding protein α (C/EBPα) and GATA-2. C/EBPα is expressed at high levels in GMP, together with low levels of GATA-2. Enforced expression of GATA-2 in these progenitors gives rise to eosinophil differentiation, while a concomitant decrease in C/EBPα and increase of GATA-2 engenders bipotent basophil/mast cell precursors. Their terminal differentiation into basophils requires C/EBPα reactivation, while its continuous diminution leads to the emergence of mast cells. As illustrated in *figure 1*, final basophil commitment seems to depend not only on the amount of transcription factor expressed, but also on the sequence of expression. Indeed, both eosinophils and basophils derive from GMP and express high levels of C/EBPα and GATA-2, but in contrast with eosinophils, terminal basophil differentiation does apparently depend on the loss of C/EBPα expression (basophil/mast cell precursors) before reactivation (basophil precursors). Although these two transcription factors seem to play a preponderant part in the fate of these myeloid cells, the mechanism through which their expression is regulated remains unclear. However, the *in vivo* effect of a short-term IL-3 treatment of bone marrow cells, directing GMP towards the basophil differentiation pathway in a STAT5-dependent manner and increasing the number of bipotent basophil/mast cell progenitors in the spleen, has recently been documented [31], highlighting once again the role of this cytokine in basophil development during immune responses.

MORPHOLOGICAL AND PHENOTYPICAL CHARACTERISTICS OF BASOPHILS

Basophils appear as mononuclear cells in human blood, measuring 10-14 μm in diameter, with a lobulated nucleus and round, basophilic granules in the cytoplasm. They exist in numerous animals from fish to mammals, with some variations as to the form of the nucleus and granular density. They are usually present in small numbers in the blood, except in turtles [2], which have a relatively large circulating population. In mice, their granular content is relatively low, at least in basophils isolated from bone marrow or lymph nodes [15, 32].

Until recently, research on basophils, and more particularly murine basophils, suffered from the lack of surface markers for reliable sorting. Even though specific antigens have still not been identified, murine basophils can now be purified after staining with a combination of antibodies defining the phenotype $DX5^+(CD49b^+)FcεR\text{-}Iα^+c\text{-}kit^-CD3^-CD11b^+NK1.1^-Thy1.2^+CD11c^-2B4^+B220^-Gr1^-$ [33]. This population can be sorted either directly from freshly isolated bone marrow cells, or expanded during eight days of culture in the presence of IL-3, giving rise to a cell population that comprises 30-40% basophils.

Basophils express a variety of membrane receptors through which they can respond to many exogenous stimuli. These sites of interaction comprise receptors for several cytokines and chemokines, Fc immunoglobulin, complement, formyl peptides, urokinase plasminogen activator, as well as leukocyte imunoglobulin-like receptors and adhesion molecules. On the other hand, basophils constitute an important source of mediators, including histamine, PAF, LTC4, granzyme B, retinoic acid, and several chemokines and cytokines [30, 34-40] *(figure 2)* and express IL-4 and

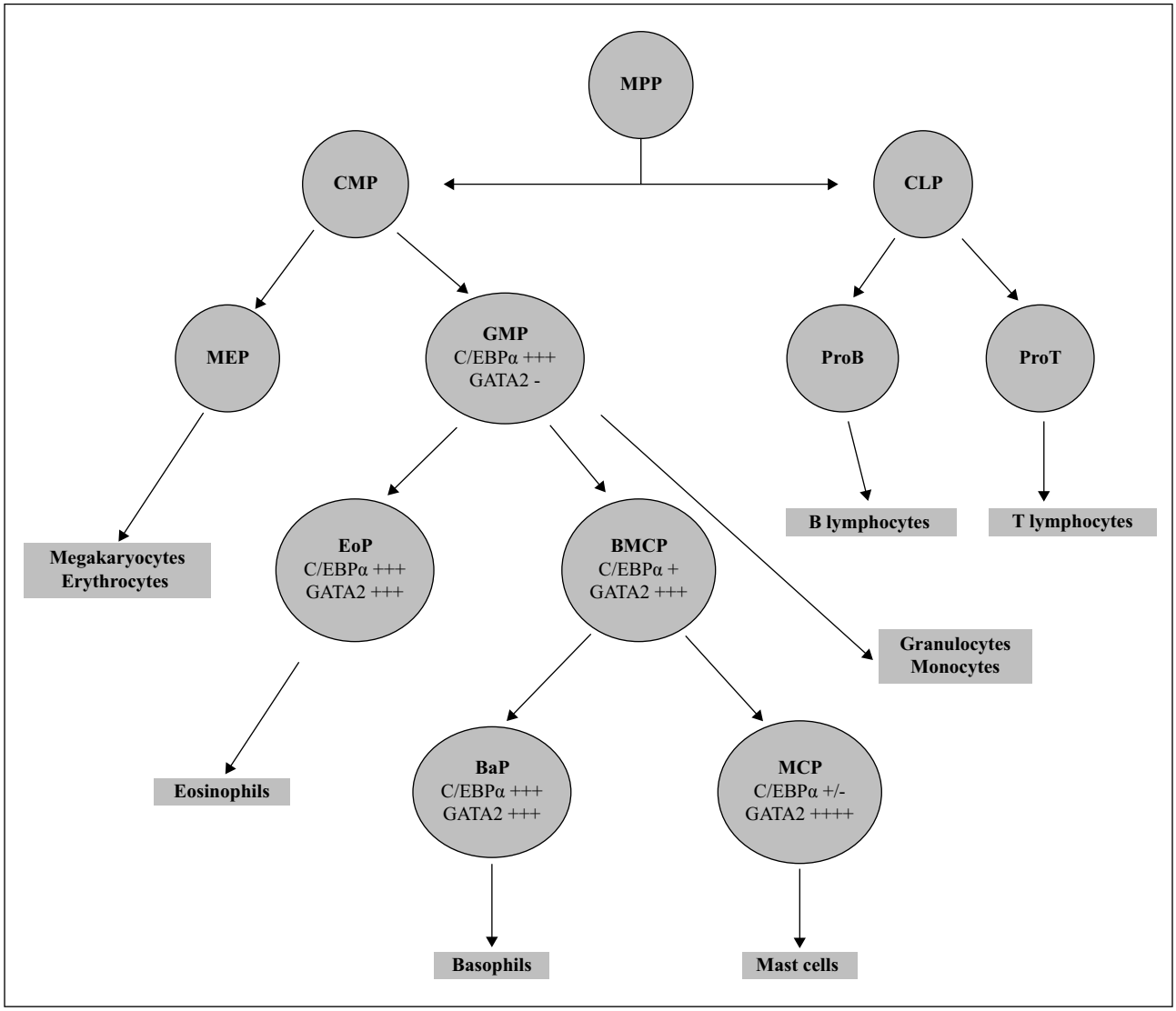

Figure 1

Schematic representation of basophil/mast cell/eosinophil differentiation pathways (adapted from [29]).
MPP: multipotent progenitors; CMP: common myeloid progenitors; CLP: common lymphoid progenitors; GMP: granulocyte/monocyte progenitors; MEP: megakaryocyte/erythrocyte progenitors; EoP: eosinophil precursors; BMPC: basophil/mast cell precursor cells; BaP: basophil precursors; MCP: mast cell precursors.

IL-13 transcripts constitutively during their ontogeny. These preformed mRNAs explain the prompt generation of these cytokines upon stimulation [41]. Taken together, these features provide a more reliable definition of basophils evocative of their regulatory and effector functions rather than purely morphological criteria that can vary from one species to another and as a function of the state of activation [32].

BASOPHILS, CYTOKINES AND T_H2 TYPE IMMUNE RESPONSES

Since 1999, it is generally acknowledged that basophils are efficient IL-4 producers [5, 11, 42]. In mice, this activity had actually been reported earlier, but was attributed to a "histamine-producing cell" or a "nonT-nonB cell" in bone marrow or spleen, respectively [6, 43], as basophils were not thought to exist in this species, although they are now recognized as the most potent source of IL-4 [12-14].

IL-3 is considered to be one of the most effective basophil stimuli, inducing IL-4 either directly or priming the cells for this activity in mice and humans [5, 44, 45]. As a rule, it signals through its specific α receptor subunit coupled with the common β_c chain. However, the FcRγ has recently been identified as a constitutive component of the IL-3 receptor, associated with the β_c chain through the transmembrane portions, in a manner distinct from its interaction with FcεRI or FcαRI. This ITAM-dependent coupling is required for IL-3-induced IL-4 production by basophils, but not for their proliferation [46]. It is reminiscent of leukocyte imunoglobulin-like receptor 7 (LIR7)-induced IL-4 production by basophils, inasmuch as LIR7 has a truncated cytoplasmic domain with a charged arginine residue in its transmembrane portion, through which it associates

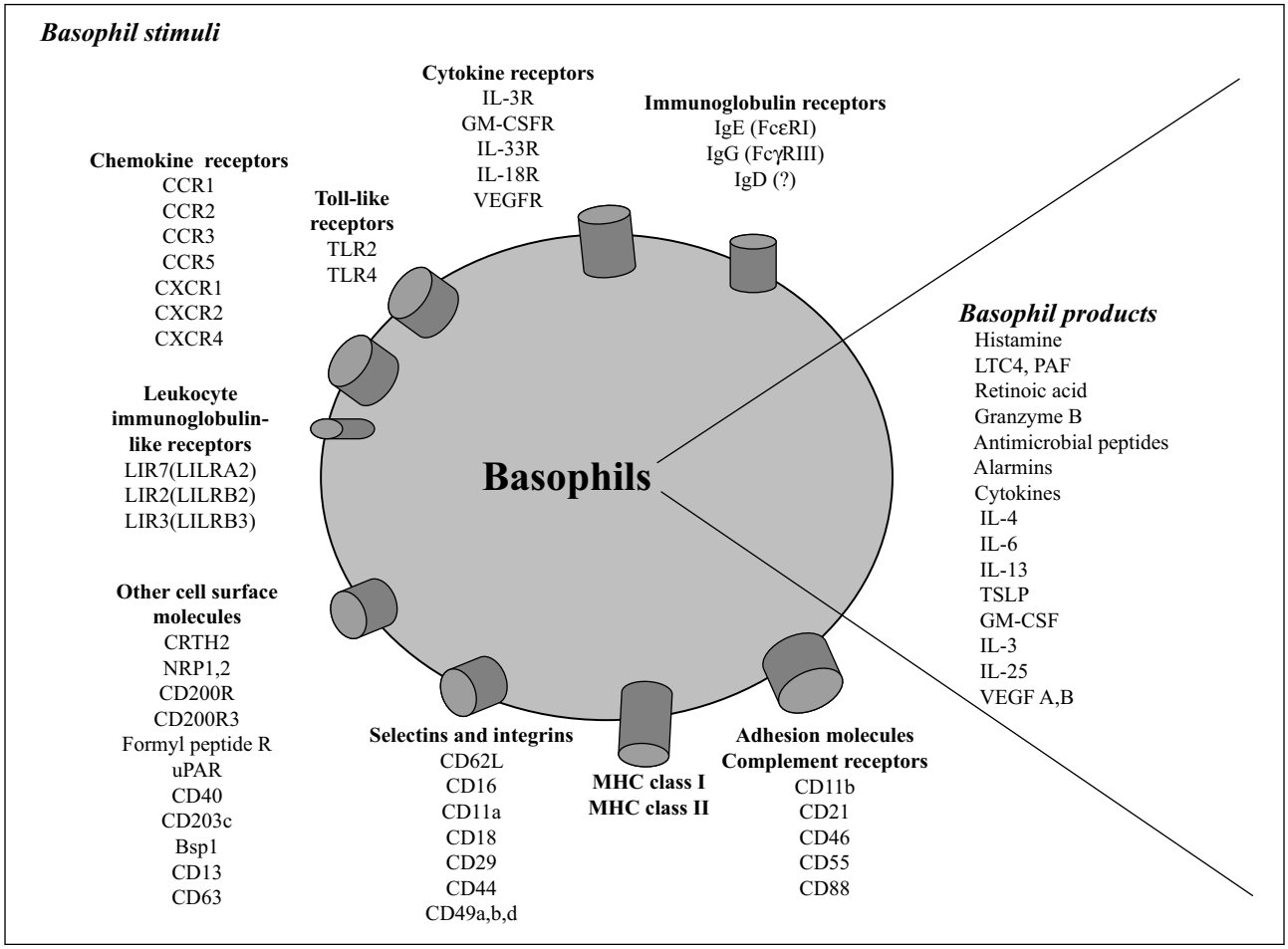

Figure 2

Basophil receptors and products.

with the immunoreceptor tyrosine-based activating motif (ITAM)-containing common FcRγ chain for signal transduction *(figure 3)* [47, 48].

The belief that IL-4 production by basophils depended strictly on FcεRI crosslinking by IgE/allergen complexes has, for a long time, been the main argument against their contribution to T_H2 polarization. Indeed, the generation of IgE depends itself on IL-4, leading to a "vicious circle", as the stimulus depends on the product it is supposed to induce, (the chicken and egg paradox). This situation has been resolved by the discovery of a number of stimuli that promote IL-4 production in an IgE-independent manner, such as:

– "superallergens", represented by bacterial, viral or other pathogen patterns that can crosslink the FcεRI in the absence of specific IgE [49];

– cytokines like IL-3, GM-CSF, IL-33 and IL-18 [7, 20, 42, 50, 51];

– proteases derived from allergens and parasites [15];

– TLR ligands, mainly TLR2 and TLR4 [52, 53];

– cross-linking of cell surface receptors, such as CD200R3 [54] and LIR7 [47] *(figure 4)*.

The first direct evidence for the contribution of basophils to T_H2 polarization of the immune response was provided by Sokol *et al.* [15] who showed that immunization against cysteine proteases, such as papain and bromelain, induces a T_H2 immune response, mediated through IL-4 produced by basophils that migrate into the T cell zone of draining lymph nodes. The preservation of an intact enzymatic activity is essential for the effect of these proteases, suggesting that they cleave a cellular sensor, which sets off the pro-T_H2 activity. Protease-activated receptors (PARs) would be a plausible target for this purpose, but in contrast to various other hematopoietic cells, there is no evidence of PAR expression in basophils as yet [55]. Several publications have subsequently confirmed the ability of basophils to polarize the immune response to allergens or helminths (*Trichuris muris* for example). Among these, three recent studies have further demonstrated that basophils can present antigens to T cells in a MHC Class II-dependent manner [16-18], and take part in the initiation and polarization of the immune response, not only through their production of IL-4, but also of other cytokines. Indeed TSLP, which has been implicated in the development and progression of T_H2 cell differentiation and allergic inflammation in both humans and mice [15, 56, 57], has also been detected in basophils, at least in those that infiltrate murine draining lymph nodes at the onset of a T_H2 immune response. This is also true for IL-25 (also called IL-17E) that contributes to the allergen-induced T_H2 profile by stimulating IL-4 production either directly by naive T cells or indirectly through another, not yet clearly defined, population [58, 59]. IL-25 also enhances the expansion and functions of

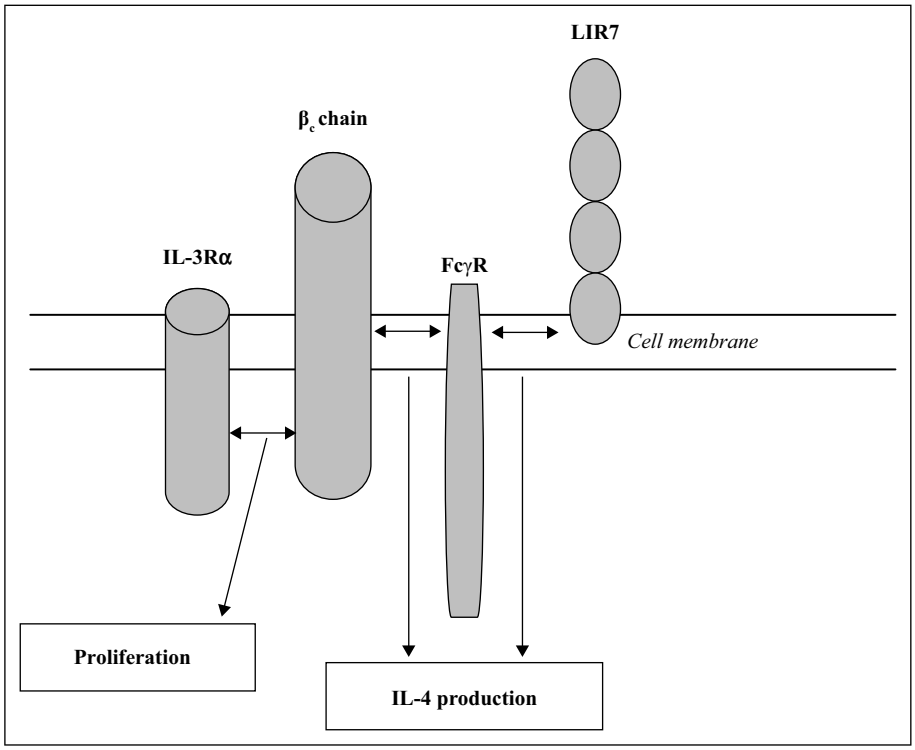

Figure 3
FcγR-dependent IL-4 production by basophils in response to IL-3 or LIR7.
Through its transmembrane portion, ITAM-bearing common FcRγ associates with the common β chain of the IL-3 receptor and with truncated activating LIR7. This association is required for IL-4 production, either in response to IL-3 or cross-linking of LIR7, while it is dispensable for IL-3-induced basophil proliferation.

TSLP-DC-activated T_H2 memory cells that express high levels of IL-25R [60]. Lastly, the effect of basophils in this context can be amplified by IL-33, either directly by promoting their pro-T_H2 cytokine production or by inducing IL-3 and GM-CSF, which in turn increase basophil survival and IL-4 production in an autocrine fashion [61] *(figure 5)*.

This pro-T_H2 effect of basophils explains why in genetically engineered mice, such as $IRF2^{-/-}$ and $Lyn^{-/-}$ strains, their increased incidence under steady state conditions is associated with a spontaneous T_H2 phenotype [62, 63]. Conversely, in accordance with the hygiene hypothesis, which claims that the prevalence of infectious diseases and allergic reactions are inversely correlated, we found that during a T_H1 response revealed by IFN-γ and FasL production by activated T and NK cells, basophils undergo apoptosis [64].

The expulsion of most gastrointestinal helminthes relies essentially on the development of an efficient T_H2-type immune response along with IL-4 and IL-13 production, a process in which basophils have been clearly implicated. Indeed, they can present antigens to $CD4^+$T cells in a pro-T_H2 context associated with IL-4 production during *Trichuris muris* infection or injection of *Schistosoma mansoni* eggs [17, 65]. They can also promote systemic eosinophilia, induce alternatively activated macrophage differentiation and contribute to *Nippostrongylus brasiliensis* expulsion after a primary infection [66]. Furthermore, they protect mice during reinfection with hookworms independently of mast cells and memory T_H2 cells [67]. The importance of basophils in setting off the T_H2 response in various models, does not exclude the implication of other cells in this process, as assessed by the development of a T_H2-type response during *Nippostrongylus brasiliensis* infection in the absence of basophils, although even in this model, they are transiently recruited to the lymph nodes in response to endogenous IL-3 [68].

In addition to their effect on $CD4^+$ T helper cell polarization, basophils have recently been shown to take part in $CD8^+$ T cell differentiation by presenting or cross-presenting antigen in a MHC class I-dependent manner to the naive population, and by promoting its conversion into the IL-10-producing phenotype through IL-4 and IL-6 [69]. Although no known regulatory or suppressive functions have been ascribed to this cell type so far, these data provide additional support for the important immunomodulatory potential of basophils.

BASOPHILS, ALLERGY AND ASTHMA

It is well established that basophils, as well as mast cells, exert effector functions during allergic responses and cause most of the typical clinical symptoms through the numerous compounds produced and released upon crosslinking of FcεRI during the immediate or late phase reaction following allergen exposure. This applies to histamine, leukotriene C4, PAF, cytokines (IL-4, IL-13, IL-6 TSLP, IL-25, TNF-α) as well as chemotactic factors that recruit multiple immune cells to the site of inflammation. By contrast, the contribution of basophils

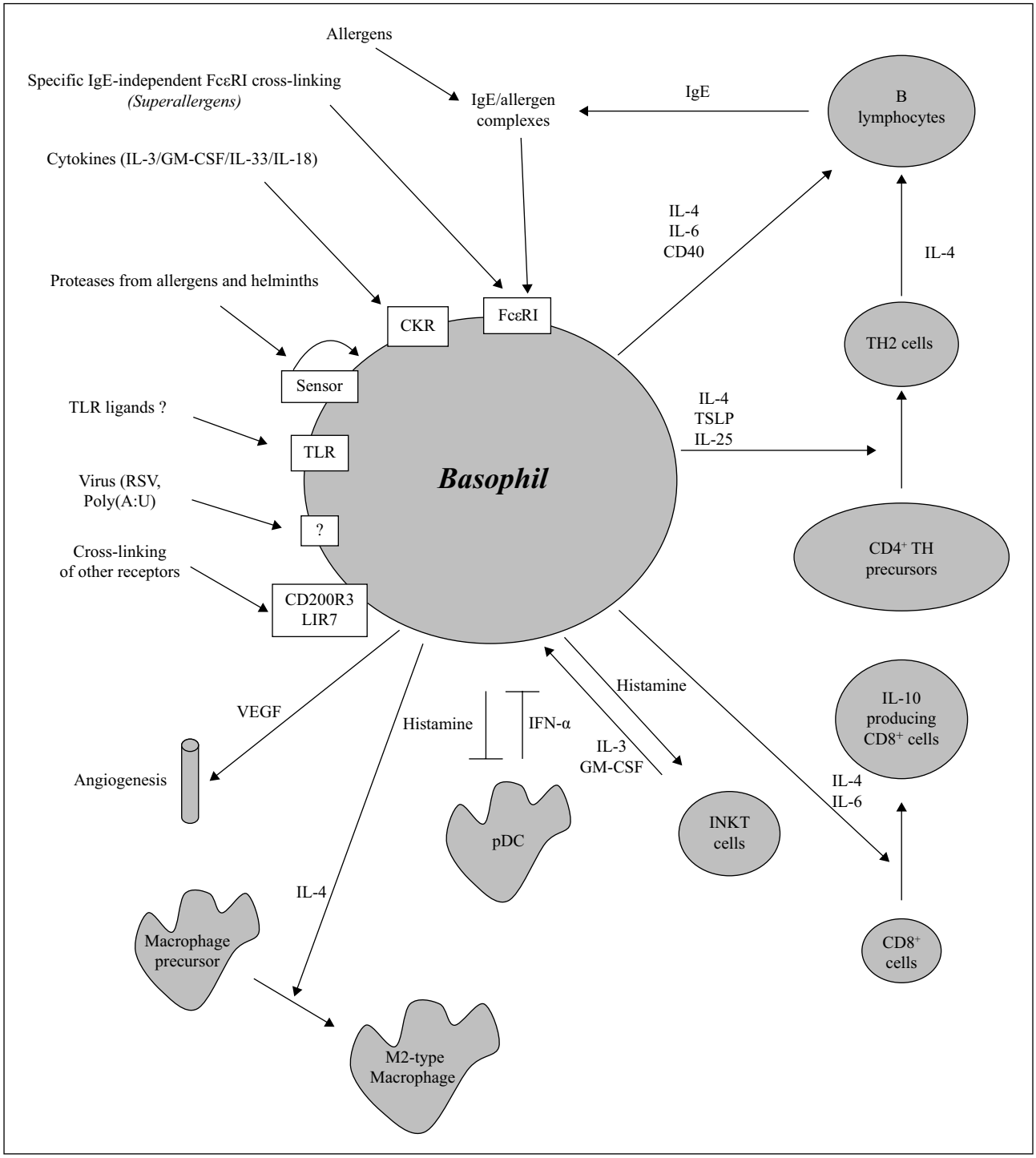

Figure 4

Immunoregulatory functions of basophils.
FcεRI-depependent and -independent stimuli induce cytokine production by human and murine basophils mediating their immunoregulatory functions in $CD4^+$ and $CD8^+$ differentiation, B cell proliferation, immunoglobulin production, M2 macrophage skewing and angiogenesis. In addition, basophils interact both ways with iNKT and plasmacytoid dendritic cells (pDC) cells. On the one hand, iNKT cells produce IL-3 and GM-CSF, which target basophils to generate histamine that is required for optimal NKT cell-derived IL-4 production. On the other hand, cytokine synthesis by basophils is hampered by IFN-α generated by pDCs, which in turn, are inhibited by basophil-derived histamine.

to the chronic phase of allergic reactions that occurs days after the initial event, as in asthma and allergic dermatitis, has remained controversial until recently. However, in 2005, Mukai *et al.* [33] established that, independently of mast and T cells, basophils promote the development of IgE-mediated chronic allergic inflammation, a process characterized by massive infiltration of eosinophils following the injection of allergen into the ear of mice carrying the allergen-specific IgE transgene. Note that the intervention of basophils is particularly effective since they account for less than 2% of the infiltrating cells. In humans, the implication of basophils in the asthmatic response has also been arduous to prove. Using a basophil-specific monoclonal antibody (2D7), infiltrating

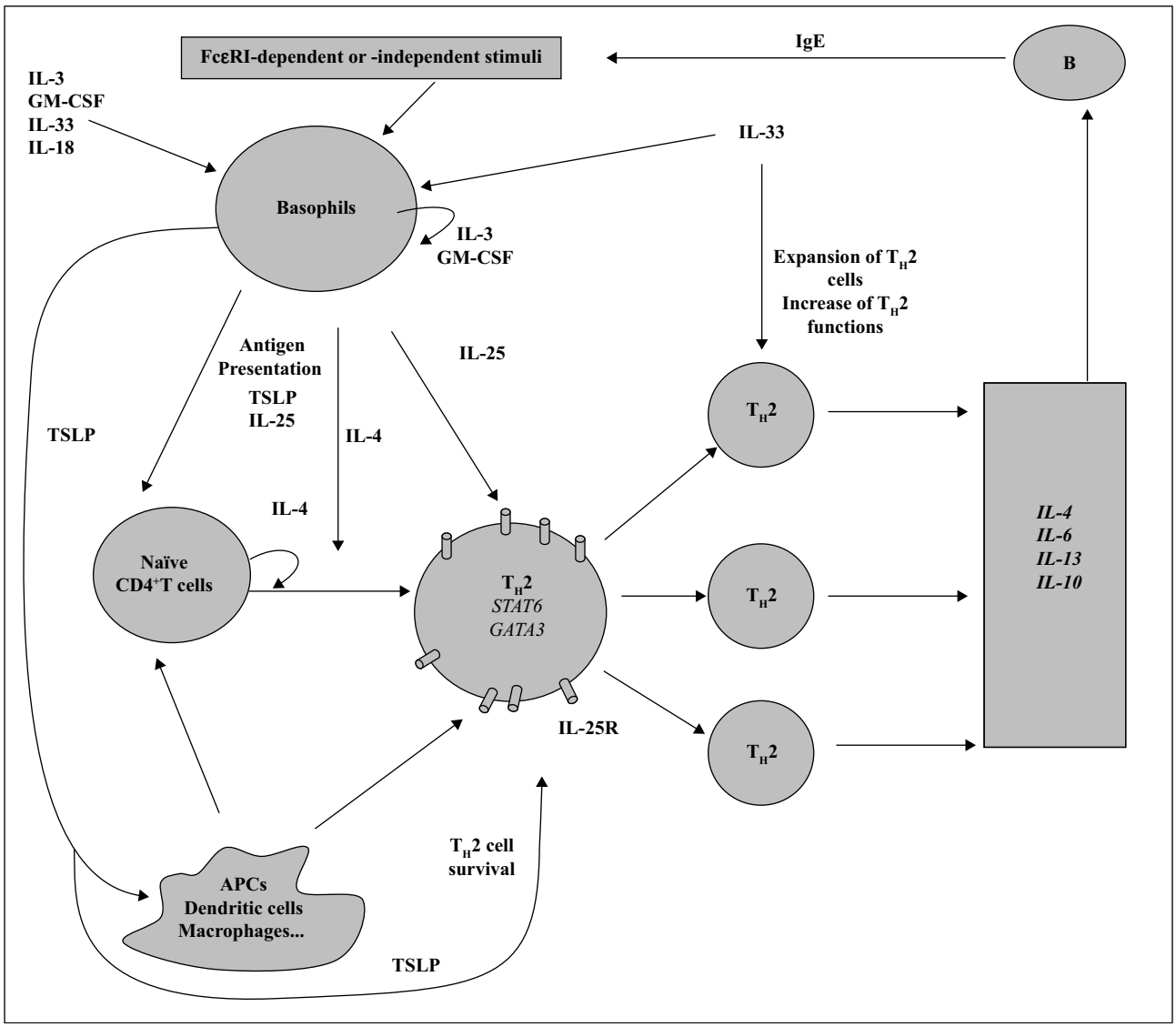

Figure 5

Role of basophils during onset and development of the T_H2-type immune responses. Activated basophils present antigen to naive $CD4^+$ T cells and produce IL-4, TSLP and IL-25 that directly or indirectly promote T_H2 polarization. TSLP-induced antigen-presenting cells upregulate IL-25R expression on the surface of differentiated T_H2 cells, which, in response to the adequate cytokines produced by basophils, amplify T_H2 proliferation and cytokine production. IL-33 targets T_H2 cells directly to increase their cytokine production, and indirectly by stimulating basophils to produce pro-T_H2 cytokines as well as IL-3 and GM-CSF that act in an autocrine manner to enhance their functions.

basophils have been detected in lung sections of patients who have died from asthma, suggesting that they contribute to the exacerbation of the disease and its fatal outcome [70]. Similarly, recent data attest that basophils are not only present in the lung during asthma exacerbation, but are also activated, as demonstrated by the upregulation of CD203c expression that returns to normal during remission [71]. In the murine system, the IL-4-producing cells emerging in the lung after antigen challenge have been identified as basophils [72]. Using the OVA-induced experimental allergic asthma model, we have likewise observed pulmonary basophil infiltration after enhancing the asthmatic response by exposure to viral products, such as ds RNA mimicked by polyadenylic:polyuridylic acid (poly(A:U)) [73]. These data support a participation in viral infection (rhinovirus, respiratory syncytial virus…), that are one of the main causes of asthma exacerbation both in children and in adults [74-76]. They open new therapeutic perspectives for its prevention. Even though the exact mechanisms leading to aggravated symptoms remain to be determined, the involvement of IL-4 is supported by the accumulation of IL-4-producing basophils in the lung during murine respiratory syncytial virus infection [77], and by the association between human IL-4 or IL-4R gain of function polymorphisms and asthma exacerbation [78]. Thus, the recruitment of basophils to the lung during fatal asthma, together with their role as potent IL-4 producers, need to be considered in future therapeutic approaches.

Anaphylaxis is a rapid, life-threatening allergic reaction that results from the binding of allergen to specific IgE bound to the FcεRI on the surface of mast cells. Upon crosslinking, mast cells become activated and secrete a

variety of mediators that are responsible for the development of the anaphylactic syndrome. However, it is now well established that FcRγ-dependent anaphylatic reactions are engendered in mast cell- and FcεRI-deficient mice. Indeed IgG, and more particularly its IgG1 subclass, can promote anaphylaxis in response to specific allergen injection. In this context, the low affinity FcγRIII, as well as platelet-activating factor (PAF), seems to play major roles [79]. Although macrophages have been claimed to be essential in IgG-induced anaphylaxis [80], recent data have provided arguments for a critical implication of basophils, as attested by their proficiency in capturing allergen/IgG1 complexes through their membrane FcγRIII, followed by PAF production. In further support of their predominant role, depletion of basophils attenuates IgG1-mediated anaphylaxis and rescues mast cell-deficient mice from anaphylactic death, while macrophages, NK cells, neutrophils and eosinophils have little or no effect on this outcome [79].

BASOPHILS AND B CELLS

Basophils express CD40L and produce cytokines (IL-4 and IL-13) that come into play during B cell activation and immunoglobulin class switching. Consequently, upon adequate stimulation, basophils can target B cells to synthesize IgE and IgG4 [81], in contrast to mast cells. More recently, it has been demonstrated that basophils contribute to humoral immune responses in several ways since they can bind antigen at their surface, are the main producers of IL-4 and IL-6 in spleen and bone marrow following restimulation with a soluble antigen, and increase the B cell helper functions of $CD4^+$ T cells [82]. Notwithstanding their low number, their frequency in the spleen (0.1 to 0.5% of leukocytes) correlated perfectly with that required to support B cell proliferation *in vitro* (0.1% of the total cell population). Depletion of basophils results in a much lower humoral memory response and greater susceptibility of immunized mice to sepsis.

Immunoglobulin D is a recently evolved Ig isotype with enigmatic functions. Like IgM, it is one of the first isotypes produced during B cell ontogeny. When immature B cells leave the bone marrow to colonize secondary lymphoid organs, they acquire surface IgD of the same specificity as surface IgM. During maturation, after antigen stimulation, B cells lose their IgD expression and switch from IgM to IgG, IgA or IgE isotype expression. However, some B cells become IgD^+IgM^- plasma cells, releasing large amounts of IgD, such as human upper respiratory mucosa B cells. This circulating IgD interacts with basophils through a receptor that induces calcium influx, antimicrobial factors, opsonising, inflammatory and immunostimulatory mediators, as well as B cell-stimulting factors [83].

FUNCTIONAL REGULATION OF BASOPHILS

The variety of immunomodulatory basophil functions prompted the search for potential inhibitors or regulators with a view to future therapeutic approaches. None of the classical inhibitory cytokines such as IFN-γ, IL-10 or TGF-β, have been reported to have such an effect, with the exception of IFN-α that impairs IL-3 priming for human basophil cytokine secretion [84] or histamine release after *in vivo* treatment [85]. However, several pathways leading to the inhibition of basophil functions, mainly in terms of cytokine production, have recently been reported, namely:

– leukocyte Ig-like receptor (LIR3) signaling that becomes inhibitory upon co-ligation with LIR7 or FcεRI, decreasing IL-4 production, histamine release and CysLT generation by basophils [47];

– the SHIP pathway that represses IL-3-induced IL-4 secretion by basophils [45];

– the inhibition by flavonoids, such as fisetin or lutein, that decrease IL-4 and other T_H2-type cytokines produced by basophils [86, 87];

– STAT1 signaling that regulates IL-4 production by pulmonary basophils in a model of primary respiratory syncytial virus infection [77];

– the lyn pathway that controls IL-4 production by basophils [63];

– prostacyclin or PGI_2 that inhibits cytokine production by basophils without affecting histamine synthesis (personal, unpublished data).

In addition to these pathways, a negative feedback can be exerted by histamine itself to inhibit IL-4, IL-6, IL-13 and its own synthesis by basophils, once its concentrations in the microenvironment have attained a critical level [88]. Indeed, basophils synthesize and release large amounts of histamine along with IL-4, IL-6 and IL-13 in response to various stimuli, dependent on FcεRI or not (IL-3, GM-CSF, IL-33, IL-18, IgE, calcium ionophore, TLRs, viruses…). They can also bind radiolabeled histamine through a mechanism that does not involve classical H1, H2, H3 and H4 receptors. It results from internalization through the organic cation transporter 3 [88] that leads to increased cytosolic concentrations of histamine that trigger a negative signaling pathway to inhibit the transcription of IL-4, IL-6 and histidine decarboxylase (the histamine-forming enzyme) genes. Even though the exact mechanism through which this occurs has not so far been determined, it is reminiscent of the effect mediated through Hic (intracellular histamine receptor) reported several years ago, and might involve the cytochrome P450 cascade [89, 90], as proposed for Hic.

Two distinct mechanisms can lead to a sufficient increase in cytosolic histamine to inhibit cytokine and histamine synthesis in murine basophils, namely uptake of histamine from the microenvironment or inhibition of OCT3 which, being a bidirectional transporter, leads to decreased histamine release following basophil stimulation and a subsequent intracellular accumulation. We have now established that other amines, such as serotonin and related compounds mimic the effect of histamine when transported *via* OCT3, thus opening the way to pharmacological manipulation of basophil functions *(figure 6)*. Some of these compounds have already been tested *in vivo* for their inhibition of basophil-dependent T_H2 cell differentiation with positive preliminary results (personal unpublished data).

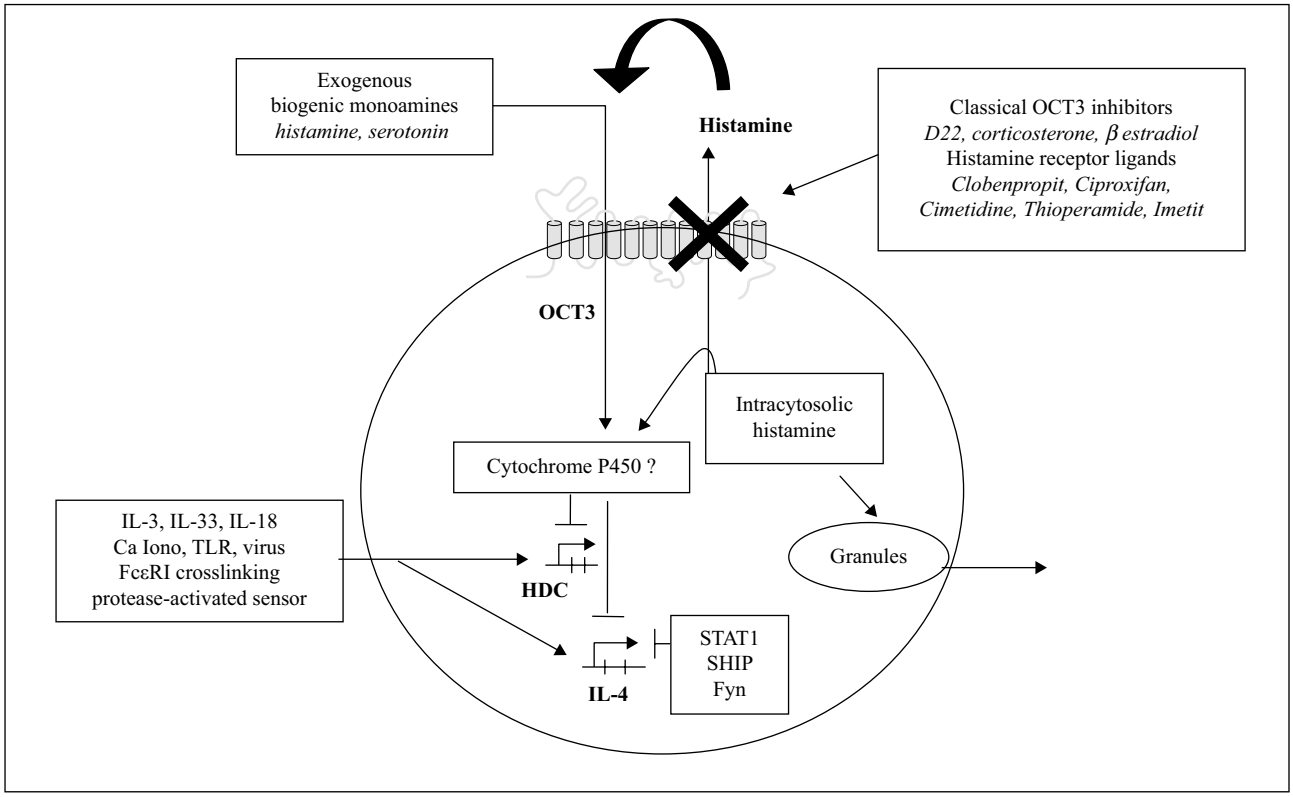

Figure 6

Regulation of basophil cytokine production. In addition to STAT1, SHIP and Fyn that inhibit IL-4 mRNA expression in stimulated basophils, histamine downregulates basophil functions through a negative feedback mechanism. In the presence of large amounts of histamine in the microenvironment, organic cation transporter 3 (OCT3) internalizes the amine that inhibits the transcription of IL-4 and other cytokine (IL-13 and IL-6) genes, most likely through a cytochrome P450-dependent pathway. Given its bidirectional function, the blockade of OCT3 by classical inhibitors or some histamine receptor ligands decreases the secretion of newly synthesized histamine, which results in the accumulation of intracytosolic histamine that signals downregulation of basophil functions similarly to high amounts of exogenous histamine.

CONCLUSION: BASOPHILS, A NEW IMMUNOREGULATORY POPULATION AFFECTING INNATE AND ADAPTIVE IMMUNITY

After many years of disregard by immulogists, basophils must henceforth be taken into consideration as a new type of immunoregulatory cell. Indeed, in addition to their well-known effector functions during the late phase of allergic reactions they exert non-redundant activities that contribute to the development of a T_H2-type immune response. These features result from their ability to generate pro-T_H2 cytokines (IL-4, IL-13, TSLP…), and to present antigen in the draining lymph nodes into which they migrate in an IL-3-dependent manner. Moreover, basophils interact, often in a bidirectional manner, with various immunocompetent cells during innate or adaptive immunity as depicted in *figure 4*, with the following activities:

– antigen presentation to $CD8^+$ T cells and induction of their IL-10 production, with unknown regulatory consequences;

– contribution to humoral memory B cell responses by providing IL-4 and IL-6, as well as help to CD4 T cell-induced B cell proliferation and immunoglobulin production;

– participation in the IL-3- and GM-CSF-induced skewing of macrophage differentiation towards the M2 phenotype;

– interaction with iNKT cells, which produce IL-3 and GM-CSF, while basophils respond to these cytokines by synthesizing histamine that is required for their optimal IL-4 production [91, 92];

– a role during angiogenesis through the production of several forms of VEGF and their receptors [93, 94];

– establishment of a basophil/plasmacytoid dendritic cell (pDc) axis, entailing a mutual functional modulation [95], such as the inhibition of human basophil functions by pDC-derived IFN-α *versus* suppression of IFN-α production by pDCs by basophils, most likely through their histamine production [95, 96].

The range of these immunoregulatory activities explains why their pharmacological modulation by specific inhibitors or regulators is actively sought for in particular, in the field of allergic diseases. Such tools may become useful in the near future in other pathologies, such as autoimmunity, as implied by the recently reported demonstration of basophil activation by anti-citrullinated protein antibodies found in the serum of rheumatoid arthritis patients [97], and the development of autoimmune disease in lyn-deficient mice [98, 99], a genetic deletion that results in T_H2 skewing through basophil activation [63].

Although clinical applications derived from these recent data are still to come, and even if "it is not because a dog can sing that it is a diva" [100], the beginning of the 21st century has already engendered enough data to change our understanding of basophil biology completely [101], bringing these cells into "the spotlight at last" [102].

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