HUMAN VACCINES & IMMUNOTHERAPEUTICS
2018, VOL. 14, NO. 4, 815–831
https://doi.org/10.1080/21645515.2017.1417711

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REVIEW

The role of basophils as innate immune regulatory cells in allergy and immunotherapy

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ABSTRACT

Basophils are circulating cells that are associated quite exclusively with allergy response and hypersensitivity reactions but their role in the immune network might be much more intriguing and complex than previously expected. The feasibility of testing their biology *in vitro* for allergy research and diagnosis, due fundamentally to their quite easy availability in the peripheral blood, made them the major source for assessing allergy in the laboratory assay, when yet many further cells such as mast cells and eosinophils are much more involved as effector cells in allergy than circulating basophils. Interestingly, basophil numbers change rarely in peripheral blood during an atopic response, while we might yet observe an increase in eosinophils and modification in the biology of mast cells in the tissue during an hypersensitivity response. Furthermore, the fact that basophils are very scanty in numbers suggests that they should mainly serve as regulatory cells in immunity, rather than effector leukocytes, as still believed by the majority of physicians. In this review we will try to describe and elucidate the possible role of these cells, known as "innate IL4-producing cells" in the immune regulation of allergy and their function in allergen immunotherapy.

ARTICLE HISTORY
Received 26 September 2017
Revised 23 November 2017
Accepted 10 December 2017

KEYWORDS
basophils; immunotherapy; T cell; immunomodulation; T reg; allergy; immune modulators; vaccinology

Introduction

Overview on the origin and main function of basophils

Basophils are polymorhonucleate leukocytes considered, with eosinophils, the major circulating immune population of effector granulocytes in allergy.[1-3] Their haematopoietic origin is still controversial. As recently reported in mouse models, both basophils and mast cells, which share functional similarities such as the expression of the high affinity IgE receptor (FcεRI) on their membrane and the involvement in allergy and hypersensitivity reactions,[4,5] derive from a common haematopoietic precursor, while their separated differentiation occurs via the differential expression of specific transcription factors.[6-8] According to recent investigations in murine models, STAT5, P1-RUNX, GATA1, GATA2 and C/EBPα are involved in basophil differentiation while STAT5, GATA1, GATA2, FOG-1 and MITF are fundamental for the mast cell commitment.[7] According to Arinobu et al., the different interplay in both the dosage and time of expression of the transcription factors C/EBPα and GATA-2 regulates the fates of mast cells, basophils and eosinophils from their myeloid precursors. The up-regulation of GATA-2 rules the differentiation towards eosinophils in the GMP, while the concurrent down-regulation of C/EBPα and the up-regulation of GATA-2 lead to the basophil/mast cell precursor (BMCP) from GMPs.[8] C/EBPα reactivation is fundamental to the basophil commitment, while the continuous suppression of C/EBPα leads to the mast cell differentiation.[8] Other studies revealed that the balance between GATA-2 and STAT5 is fundamental for basophil and mast cell differentiation and that GATA2 should act downstream of the interferon regulatory factor-8 (IRF8), which modulates several myeloid lineages, at least in mice.[9,10] The mouse model offers the possibility to investigate also the origin of basophils in humans, where the mechanism should appear much more complex and most probably the STAT5/GATA2 axis induces the up-regulation of C/EBPα for mast cell and MITF for basophil commitment.[6,11]

Actually, for a long time, basophils were functionally mismatched with mast cells, because of their shared activity in allergy, and because, likewise mast cells, they release histamine, prostaglandins and leukotrienes, are involved in innate immunity, release cytokines such as IL4 and IL13, and express FcεRI; yet, due to the complex cross talk between basophils and other immune cells, mast cells are quite far from being compared with basophils in allergy respect to other granulocytes such as eosinophils, which are more similar to basophils during an allergy response.[12]

Once released from bone marrow, basophils are usually present in the peripheral blood and in the spleen but can enter also the lymph nodes and tissue inflammatory sites, where activated cells release serine proteases (mMCP-8 and mMCP-11) and elicit an enhancement in the local micro-vascular permeability to allow T-cells and innate immune cells reaching the inflammatory site.[13-15] Circulating basophils account for

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the smallest proportion of leukocytes present in the whole blood, reaching a value that is $\leq$ 1.0% of the whole white blood cell count (WBC).[16] Their relatively easy withdrawal from venous peripheral whole blood by venipuncture allows the setting of successful assay tests for allergy diagnosis and immunotherapy, usually much cheaper and much less time-consuming than a possible assay set with tissue mast cells. Technical approaches, such as flow cytometry (FC), are able to thoroughly investigate basophil biology *in vitro*, giving the opportunity to build up a cell test (basophil activation test or BAT) considered of major importance because of the lack of specific cell lines and knock out animals available to invesigate basophil biology.[2] Basophils may be activated either by triggering the FcεRI-dependent signaling, via specific IgEs cross-linking (IgE-mediated response) or by non IgE-mediated stimuli, such as bacterial formylated peptides,[17] TLR ligands,[18] adenosine and extracellular nucleotides,[19] several cytokines and immune mediators,[3,20] xenobiotics and IgGs.[21,22] Activated basophils release a great deal of immune and vasoactive mediators, including histamine,[2,4] cysteinyl leukotrienes, which play a major role in allergic rhinitis,[23] prostaglandins, with a different COX-1 and COX-2 expression pattern between basophils and mast cells,[24] cytokines, chemokines and immune mediators, often quite different from those released by mast cells.[3,25] The pattern of released mediators does not necessarily change with the onset of an allergic or an innate immune response but basophil degranulation might be quite different in chronic allergy respect to controls.[26] Basophils can migrate in inflamed tissues, where they are recruited via the membrane up-regulation of chemokines receptors, such as CCR1 and CCR2, in response to inflammatory chemokines such as RANTES or MCP-1.[27]

From an immune point of view, basophils are circulating innate cells that not only elicit micro-vascular permeability to allow leukocytes transmigration in the inflamed tissues but also contribute in the Th2 immune response, as they are the most important $CD3^{neg}$ cells producing IL4.[1-3] Basophils participate actively in the eradication of helminths from the infected host via a Th2-mediated response, together with other innate immune cells and dendritic cells, as basophils release IL4 and IL13 and respond to further cytokines involved in the response such as IL9, IL5, IL33 and IL18.[28-30] Their involvement in innate immunity has been recently extensively reviewed. For example, murine models showed recently that, besides to IL4, basophils release also IL6, which is able to induce naïve $CD4^+$ T cells to Th17 differentiation and the response of Th17 both *in vitro* and *in vivo*.[31] Actually, the role of basophils in Th17-mediated immunity has been investigated in lung and bowel inflammatory diseases, where recent reports highlighted the involvement of basophils in the increase of Th17/Th1 cytokine expression in memory $CD4^+$ T cells.[32]

Basophil paucity in circulating blood deserves a particular attention, because it may suggest for a major regulatory role in innate and acquired immunity rather than the traditional effector function in hypersensitivity and atopic response.[33] In this perspective, new insightful data are available in the current literature focusing onto the immune regulatory role of basophils.

Background on the immune regulatory role of basophils

The very recently reported papers on basophils would suggest that these cells have mainly an immune regulatory function, both in innate and acquired immunity, rather than solely a forthright involvement in the simplest histamine releasing activity.[2,34-37]

For example, recent evidence suggest that basophil activation and development can be promoted by thymic stromal lymphopoietin (TSLP) and that probably two different functional lineages of basophils may exist, i.e. TSLP-elicited and IL3-elicited basophils.[38,39] The presumptive existence of two functional kinds of basophils, associated with their relative paucity in the peripheral blood, should lead to the consideration that basophils are much more addressed as immune regulatory cells than effector leukocytes. For certain aspects, research in allergy might be interested in elucidating if these two functional purported subpopulations of basophils could differentially affect any immunotherapeutic approach, i.e. if these cells have a differential immunological profile and/or behaviour in a specific allergen immunotherapy.[40] To tentatively reply to this fundamental question, one should turn any effort to focus onto the "immunological" role of basophils, rather than their classical activity in the allergy response. Actually, many interesting suggestions are coming from the very recent literature in the field.

During allergy, basophils are circulating cells that release various mediatior in order to address acquired immunity towards a dampening response to allergy and hypersensitivity. This immune activity occurs in tissues, where recent reports showed that the mucosal barrier, the role of the alarmin IL33 from epithelia and the microbiome play a major role in the immune activity of mast cells, basophils and eosinophils.[41] Depending on the immune microenvironment and the immune disorder occurred upon an etiopathological insult, basophils release different mediators and express different membrane markers. This should ensure these leukocytes to address several different modalities of biological response in the immune network. For example, basophils dysregulate the expression of membrane CRTH2 during chronic spontaneous urticaria (CSU), showing CRTH2 as a possible therapic target for CSU.[42] During this pathology, basophils upregulate the expression of substance P, which in turn activates degranulation and histamine release (acting as an histamine releasing factor), upregulating basophil NKR1 on the cell membrane and exacerbating CSU and the related pro-inflammatory response.[43] A major task of basophils is to orchestrate the immune response in order to dampen the causative ignition of the allergen-mediated inflammation. In doing so, they might even exacerbate the inflammatory response.

Their ability in modulating the organism's immune response has been recently reported also towards M2 macrophages, as basophils appear to regulate their differentiation.[44] Basophils activated by TLR4 or TLR2 are causative, with macrophages, of the pathophysiology of type 1 autoimmune pancreatitis[44] and interestingly this pathology is characterized by a high serum level and pancreas infiltration of IgG_4, which are notoriously induced by basophils to inhibit hypersensitivity reactions.[45,46]

Besides their immunoregulatory activity, the major effector function of basophils is to activate micro-vascular permeability

and promote a Th2 response, addressed to the production of IgE specific antibodies.[2,3] During a so called pseudo-allergic response, basophils exert mainly an immunoregulatory activity, for example, towards drugs, xenobiotics and chemicals and particularly for certain non-IgE related immediate drug hypersensitivity reactions (IDHRs) the diagnostic tools may appear quite scanty to address a therapic intervention.[47] This is probably because a non IgE-mediated response from basophils cannot be directly evaluated by the classical diagnostic approaches used for allergy and therefore it may emphasize the immune rather than the allergic role of these cells. For example, the ability of basophils to respond to bacterial derived molecules, such as formylated peptides, allows these cells to actively participate to chronic non allergic diseases, such as chronic kidney disease, where membrane markers up-regulated upon an IgE-mediated stimulus, such as CD63, are also up-regulated by fMLP.[48] Membrane markers, usually activated upon an IgE-mediated stimulation, such as CD203c, CD63, CD107a, CD193 (CCR3), CD164 and so forth, can be up-regulated also by a non IgE-mediated stimulus.[2,3] Furthermore, the marker CD300a is expressed on human basophils and, when rapidly up-regulated upon the allergic cross linking IgEs/FcεRI, inhibits basophil massive degranulaion following anaphylaxis.[49] Several markers up-regulated upon activation of basophils, such as CD203c, deserved interest as possible prognostic biomarker in non allergic immunotherapy, such as in carboplatin desensitization.[50]

During allergen immunotherapy basophils themselves may be able to dampen allergy induced by the therapy, even apparently without an immune T cell-mediated surveillance. A possible mechanism is basophil anergy. In the case of peanut oral immunotherapy (OIT) a mechanism of basophil anergy was recently described.[51] In this paper, the authors used an RPMI stimulation buffer containing 2 ng/mL IL-3, they gated basophils as $CD123^{+}CD203c^{+}HLA^{-}DR^{-}CD41a^{-}$ events in a flow cytometry (FC) approach and reported that, following peanut OIT, basophils reduced significantly the *in vitro* CD63% in FC, a reduction observed indifferently if challenging cells with specific or aspecific allergens, probably indicating an anergic hyporesponsivity.[51] According to recent views, basophil anergy should be considered as a mechanism to counteract excessive cell activation, also towards minor antigens, and it often involves the Syk tyrosine kinase down-regulation.[52] Anergy is a well known down-regulation mechanism in immune cells and it is used to modulate lymphocyte response during inflammation. The mechanism might be significantly different respect to basophil and mast cell desensitization, which can be elicited by immunotherapy with a sequential allergen challenge and hence an immune desensitization, a process that should rapidly attenuate basophil reactivity in a non-specific way by acting at a level probably upstream of the p38-MAPK signaling.[53] Furthermore, the role of IL-3 in the anergy mechanism is particularly intriguing and might even suggest that "anergic" basophils belong to a defined subpopulation of activated cells.[38,39] This intriguing aspect on anergy related to basophils does not seem to involve mast cells as well, which are considered a much more heterogeneous population of immune cells.[25] Mast cells respond to pro-inflammatory and allergenic stimuli via their heterogeneity while basophils via their ability to adaptation to different immune microenvironments. For example the ability of mast cells to release vasoactive mediators during allergy is higher than basophils, which possess less leukotriene subtypes than mast cells, although both basophils and mast cells actively participate in the innate response.[33,34]

Some cytokines enable basophils to act as immune regulatory cells. The *in vitro* incubation of basophils with IL-3 increases the expression of the beta-subunit of the basophil membrane IgE receptor, FcεRI.[54,55] The up-regulation of the beta subunit of the FcεRI competes with the subunit alpha of the same high affinity IgE-receptor, FcεRIα, so modulating the allergy response.[56] We do not know if basophils by alone might be able to account for the effectiveness of an allergen immunotherapy via the induction of anergy, yet, actually, their complex relationship with the immune system may lead to a more correct and thorough interpretation of their biology.

Is there a difference between basophil anergy and allergen desensitization at the level of membrane FcεRI-IgE-mediated signaling? Recent insights have shown that basophil anergy is a mechanism deputed to dampen unwanted aspecific reactions against minor antigens.[52] However we do not know if this is a mechanism generally adopted also during allergen immunotherapy at the level of the FcεRI/IgE membrane signaling system. Any possible response to this interesting conundrum may shed a light on the regulatory role exerted by basophils in the immune system.

At a molecular level, immunotherapy desensitization should possibly involve the Bruton tyrosine kinase (BTK). Recent reports showed an involvement of BTK in IgE-mediated allergy and the ability of BTK inhibitors to switch off the allergic stimulus.[57,58] Ibrutinib is a selective BTK inhibitor, which was successfully used for a constitutively activated BTK in B-cell malignancies and which has been recently suggested for the inhibition of the NRLP3-mediated immune response.[59] Usually, BTK activation involves the signaling mechanism mediated by Lyn and Syk, i.e. Lyn or Syk phosphorylates BTK at a critical tyrosine (Y551), which in turn activates the catalytic domain resulting in a BTK auto-phosphorylation at a second tyrosine (Y223). This mechanism has suggested some authors the use of Ibrutinib® to attenuate allergen reactivity through a molecular interference with the FcεRI/IgE-mediated signaling in basophils and mast cells.[60] Allergen immuno-therapy accounts on the ability of basophils to be down-regulated by a continuously induced allergic stimulus or a constitutive self-stimulation.[61,62] This perspective takes into account the fundamental role of basophils in the immune regulation of allergy, a role that somehow may neglect or put in a background the much more important task exerted by lymphocytes in immunity.[63] The immune regulatory role of basophils might not be a simple curiosity. Mutual interactions between T cells and basophils have been recently reviewed and further commented,[64,65] suggesting that basophils should play a fundamental role in the immune network. This should mean that the role of basophils, either in allergy or in immunotherapy, is fundamentally regulatory, a consideration that it may also suggest a kind of speculative shortcut in describing basophils as "innate T-like cells".[66]

In summary, to date we have described basophils as simple allergy effector cells. The increasing bulk of evidence and reports regarding their active participation in the regulation of the immune response, should give us the serious possibility to

thoroughly reappraise their role in immunity. Furthermore, as regard immunotherapy, a possible association between basophil anergy (as a regulatory mechanism of allergy) and allergen-mediated desensitization might exist and may serve to suggest new immuno-therapeutic approaches to counteract and prevent chronic allergy manifestations. This relationship might be further orchestrated by the participation of basophils with other immune cells, such as T cells. A possible approach to highlight this issue, therefore, is to deepen the role of basophils in the immune regulation. In the next section we will address the role of basophils as immune regulatory cells and their impact in allergen immunotherapy.

Basophils as regulatory immune cells in allergy

Background

As detailed before, basophils are still considered as circulating land mines able to exacerbate the hypersensitive response to an external (allergenic) stimulus by releasing histamine and leukotrienes. This reductive look, yet currently attributed to these highly complex cells, is loosing some impact on the most recent overviews about the role of these leukocytes in humans.[3] In the regulatory immune network, basophils have yet a major role, much more important than previously suspected.[2] For example, a mutal interplay between basophils and regulatory T cells (Tregs) in allergen immunotherapy (AIT) or specific immunotherapy (SIT) with pollen allergoids has been recently reported.[67] During the first year of SIT basophil may interact with T cells by producing leukotrienes and contributing to the recruitment and/or activation of Tregs, which increased in the second year of SIT concurrently with a reduction in lipid mediators.[67] As assessed before, the paucity of basophils within the peripheral circulating blood should suggest for a modulatory rather than an effective role of these cells in hypersensitivity and its relationship with innate immunity, while a more effective action in allergy should be associated with mast cells and eosinophils. If this is true, the role of basophils in SIT may be radically different than expected. Basophils are still used as fundamental models to assess allergen immunization and immunotherapy *in vitro*.[68] The involvement of basophils in immunotherapy has been demonstrated long time ago.[69] A renowned study from Kimura et al., showed modifications in the cell function of basophils (morphological changes, reduction in IgE and in histamine production), when challenged with housedust extracts and anti-IgE in patients with a severe form of bronchial asthma sensitive to *D. pteronyssinus* allergens.[69] These changes were further investigated and partly confirmed in the following years.[70-72] Insightful evidence suggests that a major change in basophil responsiveness to allergens administered to patients via reiterated challenges, i.e. during immunotherapy, is cell desensitization, caused by a progressive exposition to causative stimuli such as allergens or drugs. Sub-threshold desensitization leads to a loss of two fundamental signaling proteins, Syk and FcεRI, although only signaling systems, which are downstream of Syk activation, are mainly involved and are regulated by IL-3.[73] The FcεRI/IgE desensitization mechanism was observed also in mast cells and therefore represents a physiological mechanism of regulation at the IgE receptor level.[73,74] Desensitization has been considered as a fundamental mechanism underlying AIT.[75-77] The immune model involves also the skewing of an allergen-specific effector T cell to FoxP3$^+$ CD4$^+$CD25$^+$ Treg and inducible type 1 Treg (Tr1) cells.[75] According to the most common opinion, allergen desensitization in basophils should occur after years of AIT, although evidence was reported showing that, for Hymenoptera stings or airway allergens, subjects may be desensitized at the earliest stage of the allergen challenge or SIT.[78,79] Desensitization involves also a wider mechanism in the regulation of the response to an allergen, such as the involvement of allergen-specific Treg cells, causing a peripheral T cell tolerance to the allergen in AIT.[80-82] In this perspective, basophils might act as a fundamental hub in the immune regulation of allergy.

Basophils in the immune regulation and in immune disorders

An orchestrated mechanism of allergy modulation is performed by circulating basophils in association with other immune cells, via the fundamental cross-talk that they have at the crossroad of innate and acquired immunity.[83-85] For example, during rush immunotherapy (RIT) a marked reduction in the basophil-dependent release of IL4 and IL13 was recently observed.[86] A marked reduction of histamine release and of the membrane activation marker CD203c early in the treatment was also reported, with a recovery within one week following RIT.[86] Reduction in the pro-allergenic response mediated by basophils can be considered a feedback-like response of basophils to an allergen challenge, which notoriously involves the production of fundamental cytokines such as IL4 and IL13; yet, it may involve a more complex mechanism of immune regulation upon immunotherapy. During AIT with dust mite allergens, a down-regulation in IL4 genetic expression in CD4$^+$-Th cells occurs even through the IL4 gene promoter DNA methylation.[87] Dampening IL4-mediated signaling is fundamental to modulate allergic inflammation. For example, IL4 modulates regulatory T cells (Treg) activity, via the IL4Rα/STAT6 axis, so decreasing the expression of Foxp3 in Tregs and promoting allergy response.[88] Basophil modulation of releasibility, either histamine, lipid mediators or cytokines, is a way by which these cells decrease the hypersensitive response, which may lead to allergy-mediated inflammation, although advocating this mechanism as the major regulating pathway in allergy fails in elucidating how basophils directly respond to AIT. The ongoing allergen challenge, either natural or provoked (as in a SIT), should recruit basophils as major cells able to modulate allergy or alternatively dampen the same by innate immunity.

A fundamental role might be exerted by IL4, which in the case of basophils should activate and enhance a Th2-response only during allergy, while in AIT, where allergy desensitization mechanisms prevail, IL4 from basophils induces a Treg response, while at the same time basophils may contribute in inhibiting IL4 production from CD4$^+$T cells. Fig. 1 summarizes this point, trying to deepen the fundamental role of basophil in the immune regulation of allergy.

Mediators in such a mechanism include a certain amount of reactive substances, obviously encompassing also histamine.[89] It is well known that histamine released from mast cells inhibits

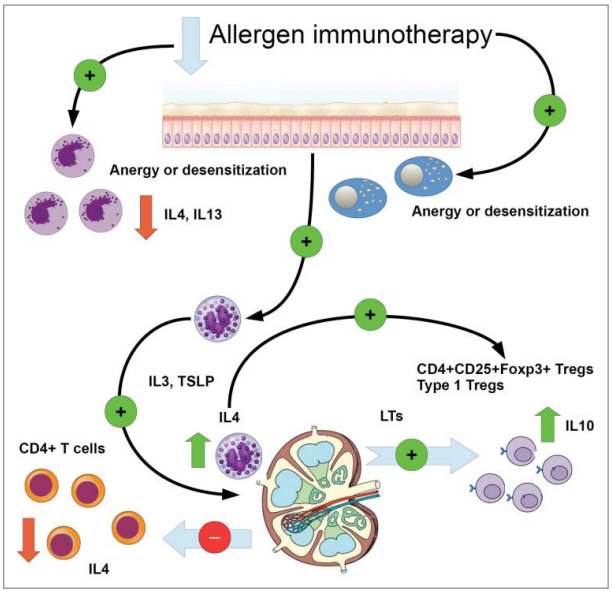

Figure 1. Cartoon showing two major assessed pathways of allergy dampening via allergen specific immunotherapy (AIT), in which basophils may be directly involved. As explained in the text, both anergy and desensitization could be a possible effect of AIT, involving both indifferently mast cells as well as basophils, probably mast cells are an early target of desensitization. Basophils reduce their release of IL4 and IL13. Alternatively, basophils may activate in lymphoid tissues via a basophil IL4-mediated signaling, the differentiation and development of Tregs and IL10-producing Tregs from naïve $CD4^+$ T cells, thus reducing the expression of IL4 in these cells and the inhibition of a Th2-mediated response. Green arrow: increase. Red arrow: decrease. Green circle: activation (+), red circles inhibition (−).

Treg suppressor function (essentially by inducing a decrease in the expression of CD25 and Foxp3 in these cells via the H_1-receptor-mediated signaling).[90]

Impairment in the activity of basophils able to induce immune disorders was rarely reported. Major data should come from mastocytosis, where anyway recent reports do not report an increased activity of peripheral basophils, despite any expectation.[91] Alterations in the basophil function have been reported in some diseases where basophils play a fundamental immune role. For example in the Kimura disease, where parotid glands are involved and a more systemic disease leading to the formation of painless masses in enlarged lymph nodes occur, basophils greatly enhance the production and release of IL4 and increase their number in the bloodstream.[92] One of the major physiological role of basophils is B cell maturation, which should lead to the specific IgE production.[93] This evidence may shed a light on why during autoimmune disease the role of basophils is imbalanced. In many sytemic autoimmune rheumatic diseases the ratio basophils/lymphocytes is lowered, respect to other granulocytes/lymphocytes ratios, assessing that basophils are most probably recruited in the lymph nodes and inflammatory sites.[94] Production of IgE does not only deal with allergy but recent findings show that autoreactive IgEs, such as dsDNA-specific IgE as occrring in systemic lupus erythematosus (SLE), are able to potentiate plasmacytoid dendritic cells (pDCs) via the FcεRI, followed by the TLR9 activation and to exacerbate SLE-related inflammation.[95-97] Basophils are particularly able to cross talk with DCs, besides to partcipate in B cell antibody switching, in a complex interrelationship with mast cells[98,99] and therefore their involvement in the etipathogenesis of autoimmune diseases is particularly intriguing if the allergic response is impaired or dysregulated. When dysregulation occurs in the modulation of the Foxo-1 expression in Treg cells, for example, the activated Treg function may lead to autoimmunity.[100]

In summary, some complex immunological pathologies involve basophils, though no allergy mechanism is directly implicated, showing that these cells are deeply related with immune modulation.

Insights on the relationship with regulatory T cells

Actually, the role of Tregs is fundamental in preventing impairment in the allergy response, even in AIT.[101] AIT should induce a specific Treg response with the purpose to abolish the allergen-caused proliferation of Th1 and Th2 cells, by releasing in the immune microenvironment IL10, TGF-β and surface molecules such as CTLA-4, PD-1, mTGF-β, m-IL10, IL10R and TGF-βR.[101] Furthermore, Tregs drive the immune response caused by allergy to the suppression of pro-inflammatory cells and via the IL10 and TGF-β skewing an isotype switching from IgE towards IgG_4 and IgA.[101,102] While mast cells inhibit Treg suppressor function via the histamine-H_1 signaling, circulating basophils might have an opposite role. During SIT for grass pollen, some authors reported an increase in the plasma levels of specific IgG and IgG_4 during the first year of SIT, while the significance of this increase was reached during and after the second year of SIT, when also a substantial enhancement in the number and activity of Tregs and a decrease in leukotrienes were observed.[67] A speculative overview of this evidence should suggest that while an early response to allergy or allergen specific immunotherapy involving mast cells dampened the Treg involvement in the immune response, the second (late) response might involve much more significantly circulating basophils, recruited in the antigen-presenting sites to modulate the hypersensitivity reaction

It is tempting to speculate that, in this circumstance, while vasoactive mediators, such as leukotrienes and histamine, have a role in promoting an immune response from tissue mast cells, the same mediators should mainly have a regulatory activity on the immune pro-inflammatory response via the basophil.[103] Within few minutes following allergen challenge, i.e. upon allergen binding on the FcεRI/IgE complex, basophils produce and release leukotriene-B4 (LTB4).[104] A past report showed that the release of LTB4 from leukocytes preceded anergy in these cells.[105] Furthermore, a more recent paper reported that both LTB4 and prostaglandin E2 (PGE2), the latter produced by basophils only upon a FcεRI, rather than a FcγRI signal,[106] are able to independently modulate the differentiation of the immunosuppressive CD4+CD25+Foxp3+ T regulatory cells and Th17. While LTB4 dose-dependently increased RORγt expression and secretion of IL-17 in Th17 cells, PGE2 exerted an opposite effect, while both LTB4 and PGE2 inhibited Treg function and Foxp3 expression.[107] This increase occurred when $CD4^+CD62L^+$ T cells (central memory T cells) were activated under Th17-promoting conditions in the presence of TGF-β1 and IL-6.[107] Interestingly, $CD4^+CD62L^+$ T cells can be converted to $Foxp3^+$ T cells by TGF-β.[108]

All these findings show that the involvement of basophils in the complex milieu of lymphocytes and other leukocytes is particularly dense. The immune "role" is exerted also by basophil released leukotrienes. For example, the LTB4-promoting differentiation of IL17-producing T cells, although apparently observed only in mice, might elucidate the involvement in allergy of the "natural" regulatory T cells respect to the TGF-β "induced" Tregs. The expression of Foxp3 in $CD4^+CD25^+$ regulatory T cells confers a set of different properties and markers to these leukocytes, in order to set them as an immuno-regulatory phenotype able to dampen allergy-related inflammation, produce IL10 and TGF-β and induce anergy. These "natural" regulatory T cells, which should account for the maintenance of immune tolerance and anti-inflammatory response, are neither terminally fixed, nor dependent from different functional immune conditions, yet they may change phenotype markers, cytokine profile and function.[109] In the absence of a $CD8^+$ signal, both "natural" Tregs (nTregs) and those regulatory T cells induced by other stimuli, such as TGF-β, ("induced" Tregs, or iTregs),[110-112] though sharing the $Foxp3^+$ suppressor phenotype, will develop in IL13 and IL17 producing T cells, respectively.[109] Initially, the induction of iTregs requires TGF-β and consequently the presence of IL-6, which should induce the development of a Th17 phenotype.[113]

Under Th17-polarizing conditions, $CD4^+CD25^+Foxp3^+$ regulatory T cells do not express an immunosuppressive or pro-tolerant phenotype but exhibit a pro-inflammatory, pro-allergic behaviour, for example by promoting and enhancing the expression of IL17A, IL17F and IL22 from $CD4^+CD44^{lo}CD62L^{hi}CD25^-$ naive T cells, which can be differentiated into Th17 cells also by IL6,[114,115] which is also produced by basophils.[18] The Treg/Th17 balance is a fundamental hallmark of regulation of allergy and it is not surprising that circulating basophils, able to access the lymphatic trafficking, actively participate in modulating this mechanism.[116,117] In allergic asthmatic patients the ratio Treg/Th17 is decreased while the expression of Notch1 mRNA increased,[118] an evidence that should assess the idea that, at the crossroad of the anti-allergic/pro-inflammatory regulation, a key role is exerted by the Treg/Th17 balance.

Actually, the overall picture is much more complex than expected.

A much more differentiated population of regulatory T cells involved in allergy has been described in recent years. Besides to $CD4^+CD25^+Foxp3^+$ natural T regs (nTregs), which include also the $CD4^+CD25^+Foxp3^+$ $ICO5^+$ inducible co-stimulator Tregs, further functional sub-populations were described as involved in allergy, such as the $CD4^+CD25^-Foxp3^+$inducible/adataive Tregs, the $CD4^+CD25^+Foxp3^-$ IL10 type 1 producing Tregs (Tr1), the $CD4^+Foxp3^+CCR6^+ROR\gamma t^+$ IL17 producing Tregs and the $CD8^+CD25^+CD28^+Foxp3^+$ $CD8^+$ Tregs.[119] What is interesting for our purposes is that Tregs actively participate in allergen specific immunotherapy and different subpopulations appear to be induced by different routes of immunotherapy, either if epicutaneous (EPIT), oral (OIT) or sublingual (SLIT).[120] EPIT induced the greatest increase of $Foxp3^+$ Tregs, while as OIT caused the increase of LAP^+ Tregs and only SLIT the induction of $IL10^+$ Tregs (i.e. Foxp3-negative Tregs, which should express GARP/LAP and, when activated, Helios instead of Foxp3).[121] In EPIT the suppressive activity depends on the expression of CTLA-4, for OIT on IL10 and CTLA4 and for SLIT only on IL10.[120] Although this evidence was reported in laboratory animals, it presumably should fit to AIT in humans. As a matter of fact, recent reports suggest a major role for extra-thymically induced regulatory T cells as fundamental target of allergen immunotherapy.[122]

Possible specific role for basophils in the immune regulation

In this complex functional network, which is the main regulatory role of basophils?

Recent papers outlined the evidence that basophils are engaged in a complex cross talk with a great deal of immune cells, B-cells, T-cells, dendritic cells, monocytes and so on.[123] Besides to pro-Th2 cytokines, such as IL4, IL13, IL25 and TSLP, basophils participate in the modulation of the innate and acquired immunity by producing IL6.[123] A fundamental evidence is that basophil-derived IL6 is a major cytokine for Th17 cell differentiation and the immune memory response.[3,31] At least in an experimental model of murine colitis, basophil-derived IL6, together with IL4, is able to modulate a T-cell driven autoimmune reaction by down-regulating in these cells the expression of IL2, IFN-γ and TNF-α.[124] The strategic role played by basophil, through IL6, in the differentiation of Th17 cells, must be of the utmost interest in the aforementioned Treg/Th17 balance and its role in the immune managing of the allergic response.[124]

Upon allergen cross-linking of the IgE/FcεRI complex, basophils release histamine, which is notoriously a mediator of inflammation.[125] Th17 cells, particularly if polarized by IL1β and IL23, express the histamine H_4 receptor, evidence that should assess the relationship between histamine producing basophils and the Th17 function.[126] Recent evidence reported that basophils are able to express MHC class II and co-stimulatory molecules, migrate into draining lymph nodes, present antigen to naive $CD4^+$ T cells and promote Th2 cell differentiation.[127] Previous reports showed that this recruitment in draining lymph nodes is most probably led by IL3.[128] In this microenvironment, basophils should exert their immuno-regulatory function. During a hypersensitivity reaction, a TLR4/MyD88-dependent recruitment of basophils into lymph nodes, (locating close to the $CD4^+$ naïve T cells), concurrently with the expression of the surface FcεRI on dendritic cells, has been observed.[129,130] Although IL-3 was reported as a key cytokine able to enhance IL4 production in basophils and to recruit them in lymphoid tissues, basophils are also induced by thymic stromal lymphopoietin (TSLP) to enter lymph nodes.[8,131] TSLP, which is expressed and released also by mast cells and basophils, orchestrates the Th17-driven immune response through the activation of dendritic cells (DCs).[123,132-135] The TSLP-mediated triggering of basophils should generate a different functional lineage of these cells respect to the IL3-elicited ones, which are able to respond to cytokines IL1, IL33 and IL18 and produce much higher levels of IL6, besides to IL4.[8,136] Interestingly, biochemical and hormonal regulators of the blood pressure, which exert a fundamental role also in immunity, can elicit TSLP and contribute substantially to the TSLP-driven Th2 or Th17-mediated responses.[135,137] This evidence allows to assoiate the role of tissue mast cells with this

basophil-orchestrated signaling network, which are quite far from the mechanisms occurring in lymphoid tissues and in the bloodstream and having a primary role in the early contact with the allergen. Besides to mast cells, also keratinocytes produce large amounts of this cytokine, as reported in atopic dermatitis-affected subjects.[138,139]

In this context, the role of TSLP in basophil activation, however, has been discussed as a fundamental mechanism of skin allergy and protease-induced asthma or, more generally, in the context of an IgE-independent allergy.[3] These basophils, able to respond to IL33 and IL18, may migrate from bloodstream to the skin, but might not have any significant role in specific immunotherapy, except for EPIT.[140]

Regulation of basphils. Insights on the basophils desensitization/anergy mechanism and relationship with immunity during AIT

Besides to the central role exerted by basophils in the immune regulation of allergy, these cells should play a major role in the control of hypersensitivity reactions, i.e. if they lead to an inflammatory response or should be rapidly dampened. Allergy desensitization must therefore initially occur at the level of basophils and mast cells. In this perspective, the role of circulating IgGs appears also fundamental. At the mast cell levels, for example, IgG-mediated allergy desensitization may occur either through the allergen neutralization or also via the FcγRIIB recruitment of the SHIP-1 phosphatase, which inhibits the FcεRI-mediated signaling. Furthermore, at least in mice, in experimental models with Fel d1 AIT, the receptor FcγRIIB enhances FcεRI internalization and inhibits mast cell activation via the blocking of calcium flux and degranulation.[141-142] Interestingly, specific immunotherapy involves the production of specific IgGs,[143,144] besides to the production of blocking IgG_4.[145,146] In EPIT, the production of IgG antibodies mediates the inhibition of basophil function.[147] Cat hair allergen specific IgGs desensitize the basophil-mediated allergy response through the involvement of both FcγRIIA and FcγRIIB,[147] a mechanism reported also in humans.[148-150] However, the mechanism of IgG/FcγR-mediated desensitization of allergy, occurring in immunotherapy, is quite different from other allergy modulating pathways, e.g. those ones involving Treg-mediated immune tolerance or basophil anergy, which, as previously indicated, should act as a barrier from negligible allergens.[52] As already indicated, some experimental evidence in SIT, suggested an anergy mechanism for basophils to explain allergy desensitization.[51]

It is fundamental, therefore, to deepen the bio-molecular mechanism and signaling underlying any event of basophil desensitization occurring following AIT. In the case of basophil anergy, which is supposed to be a physiological mechanism to reduce the impact of a hypersensitive reaction to minor allergens,[52] recent reports indicate a loss in the spleen tyrosine kinase (Syk) function, though controversial opinions and evidence were also reported.[151] This tyrosine kinase activates phospholipase C (PLC), playing a fundamental role in the FcεRI-mediated signaling. Any intracellular portion of Fc receptors contains an immuno-receptor tyrosine-based activation motif (ITAM) and tyrosine kinases as Syk add a phosphate in a Tyr residue of ITAM, generating a signaling cascade within the cell.[52,152-154] The down-regulation of both Syk expression and function has been suggested as the major molecular cause of basophil anergy, which has been described as a possible intermediate state between "naïve", non responder basophils and activated ones.[52] Some case of oral immunotherapy with peanut allergens showed possible basophil anergy lasting several months following OIT.[51]

To date, we are unable to assess if specific immunotherapy (SIT) preferentially generates anergy in basophils or a Treg-mediated response. A recent evidence showed that specific birch pollen immunotherapy caused a Bet v1-specific Tr1 cells (IL10 producing Tregs), with an enhancement in the production and release of specific IgG antibodies.[155] This mechanism should explicate the generation of blocking IgGs, able to dampen the allergic response via the FcγRs. Basophils might have a pivotal role in these events.[74,1056-158] The fundamental role in the regulation of allergy-mediated inflammation exerted by basophils was also associated with tissue mast cells, even described as Yin-Yang modulators of allergy.[159]

The role of mast cells in the finest regulation of allergy may be marginalised, however, because of the functional interaction of basophils with T and B cells in the lymphoid organs, which renders basophils able to orchestrate a complex functional network of immune cells, probably in a greater extent than tissue resident mast cells.[2,160] Actually, basophils are able, through a mechanism that is CD28-dependent and apparently IL4-independent, to induce $CD8^+$ T cells (without any Treg phenotype) in the production of IL10.[161] This is a fundamental hallmark also for skin and tissue immunology. Tissue resident memory $CD8^+$ T cells are the first line of defence against repeated insults, infections and allergen ongoing challenges.[162] The production of IL10 initiates a modulating mechanism that would finally recruit the $CD25^+$ regulatory T cells. The experiments of Kim and colleagues reported that the stimulation of ovalbumin-specific OT-I $CD8^+$ T cells with splenic dendritic cells pulsed with ovalbumin-derived peptides, induces primarily the production of IFN-γ, yet, by adding basophils into the coculture a futher IL-10 production was induced.[161]

Furthermore, basophils can present antigen to $CD8^+$ T cells and the co-stimulation with CD28, without ICOS or CD86, induced an enhancement in IL10 production.[162] IL10 is an inhibitory cytokine for $CD4^+$ T cells and antigen presenting cells (APCs) but it seems that, at least initially, IL10 stimulates $CD8^+$ T cell function, though lately inhibiting OT-1 $CD8^+$ T cell expansion (in a secondary stimulation), therefore most probably it acts as a priming cytokine for $CD8^+$ T cell activity.[163] The contribution of basophils in this sense might be to enhance the immune cell response to the allergen (antigen) in the tissues, where mast cells are addressing the allergenic agent. Moreover, some basophil-released chemokines, such as CCL3 and CCL4, are also able to recruit $CD8^+$ T cells in many inflammatory sites, including tumors.[164] Desensitized basophils, which reduced their release in IL4 and IL13, are most probably induced to dampen the $CD4^+$ T cell-mediated response and activate the initial tissue $CD8^+$ one, with or without a subsequent $CD25^+$ Treg involvement.

As reported earlier in this review, activated basophils produce mainly IL4 and IL13. Production of IL4 from basophils

can be triggered by the histamine releasing factor (HRF), induced from tissue also by monocytes through the chemokines MCP-1.[2,3] The complex HRF/TCTP (translationally controlled tumor protein) is a potent segretagogue for histamine and IL4 in basophils.[165] Furthermore, HRF/TCTP primes non responder basophils to release histamine and IL4 in the course of an allergic response.[166] This mechanism of priming is quite different from the same induced by IL3 and it is still far to be fully elucidated.[166] Priming and desensitization are two opposite mechanisms enabling cell to respond promptly to minor stimuli, usually anticipating a major functional event. It is tempting to speculate that both priming and desensitization correspond to two "bifurcation" events suited to drive cells to a defined functional decision. In this sense, they are functional modes of the complex balance involving basophils in the immune network.[167,168]

Desensitized basophils may contribute to mast cell down-regulation of allergy functional markers. At least in mice, during contact dermatitis a $CD8^+$ T cell population expressing PD-1 and having an effector memory phenotype, is able to produce IL10 in a way comparable to that of effector $CD4^+$ T cells.[169] Further studies have highlighted the mechanism underlying contact hypersensitivity and shown that an initial IFN-γ producing $CD8^+$ T cells, infiltrating the skin, characterized the first 48 hours of the hypersensitivity response, just earlier respect to the recruiting of IL4-producing $CD4^+$ T cells.[170]

Basophils produce a great deal of pro-inflammatory or anti-inflammatory (Th2-type) cytokines, which modulate the immune response to allergen, also in AIT.

Interferon gamma (IFN-γ) is produced upon IgE-mediated activation in basophils.[171] This cytokine is also produced by anergic T cells. Past evidence has shown that T cells reactive to the allergen Fel d1 peptide 4 or to the influenza A haemagglutinin peptide, become anergic or regulatory if the agonist peptide dosage is high and in the absence of APCs.[172] These cells are induced in producing IFN-γ but not IL2.[172] At least in asthmatic models, IFN-γ, produced by Th1 cells, serves as an anti-inflammatory cytokine, likewise IL12, which is produced by macrophages, dendritic cells (DCs) and B cells to regulate Th1 cells differentiation.[173] Invariant natural killer T cells (iNKT cells) are particularly sensitive to DC-derived IL12 and, at least in mouse models, they trigger basophil reduction by apoptosis, a mechanism elicited by the ability of Th1-produced IFN-γ to activate iNKT.[174] Therefore, it is particularly interesting to speculate that IFN-γ directly produced by basophils upon IgE-mediated stimulation or by the indirect activation of the $CD8^+$ T cells, should finally promote basophil reduction via apoptosis, thus reducing or lowering the allergy response.

We do not know if this mechanism is occurring also in humans, anyway. If yes, reduction of allergy may derive from either anergy or desensitization, or regulatory T cells or basophil apoptosis or any of them, in concurrent events.

Further insights are needed to elucidate this issue.

In summary, the role of basophils in the immune network is highly complex and a great deal of evidence domonstrates that these cells actively participate in modulating both innate and acquited immunity, even in allergen immunotharapy, an issue that will be addressed in the next chapter.

Basophils as modulatory cells in allergen immunotherapy

The role of basophils in immunotherapy should not apparently be so different respect to a common type I hypersensitivity reaction, yet only if we exclude some fundamental issues regarding SIT. The activity of basophils in the mucosal microenvironment, as occurring in OIT and SLIT, should be radically different respect to skin, as occurring in EPIT.

In oral immunotherapy (OIT), a food allergy-preventing approach, the production of immuno-globulins IgGs dampens the IgE-mediated response through the activity of FcγRIIB.[175] The low affinity immunoglobulin G receptor FcγRIIB (CD32) is a potent target for immunoglobulin therapy and is expressed also in B- and T cells, where it inhibits the downstream signaling cascade leading to the activation of these cells.[176,177] The receptor should be also involved in the mechanism of IgG-mediated anaphylaxis in mouse.[178] CD32 was recently indicated as a fundamental cause of inhibition of the basophil activation and degranulation as well as in mast cells biology.[179-181] The cluster of FcγRs, in mast cells as well as in basophils, includes two stimulatory receptors, i.e. FcγRI (CD64) and FcγRIII (CD16) and one major inhibitory one, i.e. FcγRIIB (CD32).[182-184] FcγRs are necessary for an IL4-mediated immune response, also from basophils, which produce and release IL4 in response to bacterial products such as lipopolysaccharide (LPS or endotoxin), IL18, IL33 and IL3, when a FcγR-Syk axis is activated.[185]

From the basophil point of view, there are few major mediators that rule the immune balancing during immunotherapy and are represented, presumably, by histamine and IL4. Furthermore, in the allergy mechanism, earliest mediators of IL3-primed basophils are leukotrienes (such as LTC4) and histamine.[186] These mediators can be produced by basophils acting as regulatory cells also during the early onset of a Th1-mediated response, when these cells produce and release type I IFNs.[187] This might assess the hypothesis that histamine is a basophil-released mediator secreted to regulate the immune response and promote the induction of immune tolerance.[188-190] During a pro-inflammatory or allergenic stimulus, basophils can arrange a set of mechanisms that should respond to allergens, then rapidly dampen the same reaction while they are mediating a wider and much more proper immune response, through the involvement of DCs and T cells. Major mediators of such response from basophils are, therefore, most probably histamine and IL4.

In addition, during oral immunotherapy, either oral (OIT) or sublingual (SLIT), the participation of secreted mucosal IgA immuno-globulins should be also considered.[191] After peanut-allergens oral immunotherapy, a dramatic increase in peanut-specific IgAs in patients' saliva, correlating with food challenge outcomes, was recently observed.[192] Interestingly, the FcαRI (CD89) is expressed in those cells co-expressing also the FcεRI, and evidence was reported about the co-aggregation of FcαRI and FcεRI in regulating basophils activation and degranulation by an intracellular modulation of signaling (via the phosphorylation of the inositol polyphosphate 5′-phosphatase SHIP1 and FcεRβ.[193] At least in mouse models, the production of mucosal IgA antibodies and the presence of lymphoid tissues, such as Peyer patch, represents distinct elements in immune tolerance to food and oral antigens.[194] Nevertheless, at least in gut, a

fundamental Treg/IgA axis has been reported to support the immune tolerance and homeostasis with microbiota in the intestinal microenvironment.[195] Therefore, the role of IgAs also in oral mucosa may be related to the maintenance of an immune tolerance, particularly in OIT or SLIT.

However, many of these experimental or even speculative suggestions about how OIT, SLIT or EPIT (or subcutaneous immunotherapy or SCIT) are really working in the immune system, still need to be further addressed. The many forwarded hypotheses, basophil anergy, basophil and/or mast cell desensitization or cell number reduction, increase in IgG_4 and humoral IgA immunoglobulins, Th cell skewing from Th2 to Th1, induction of tolerant regulatory T cells, induction of Tr1 (IL10 producing T regs) and so on, cannot account by alone to explain the great complexity underlying an AIT-dependent outcome.[196] Most probably, the continuous allergen challenge induced by AIT should induce a mechanism of rapid desensitization and of immune tolerance, leading to the positive outcome of the therapeutic approach.

The current interesting question is: why this does not happen usually in the natural course of allergy, for which AIT could be a good therapy?

We do not know if a possible reason would be found by investigating the type of allergen, its amount during challenge and its administration routes.[197] Immune tolerance was observed in OIT but requires higher doses of allergen than SLIT or EPIT, its efficacy is higher than SLIT but with more systemic adverse effects compared with EPIT or SLIT, suggesting new improving approaches.[197-201] A different set of compensatory and modulatory mechanisms are probably induced, depending on the type of AIT.[120] The different immune microenvironment in oral, gut and dermal tissues may account for the different outcome of the many kind of AIT.

Possible role of tissue innate lymphoid cells

Mast cells in the mucosal tissue of oral cavity have a pro-inflammatory phenotype.[202] Turning from a pro-allergenic to a pro-inflammatory phenotype might be a fundamental mechanism for tuning the activity from "effector" to "modulating". Basophils are also major actors of allergy-mediated inflammation and allergy-induced exacerbation of the inflammatory response.[203] They migrate in lymphoid tissues and orchestrate, with DCs and other immune cells, the Th2 cells differentiation and activation, promoting the production of Th2-type cytokines.[203] If this mechanism of allergy response regards skin, such as in atopic dermatitis (AD), basophil infiltrate and accumulate with the group 2 innate lymphoid cells (ILC2s) in the inflamed lesions of skin, via the basophil-produced IL4, which increases ILC2s proliferation and AD pathogenesis.[204]

These ILC2s have a major role in the regulation of epithelial immunity, as they localize at the functional boundaries represented by the barrier organism/external environment, such as lung, gut, skin or mucosal tissues and seem to play a Th2-like role, producing also IL13 and IL5 (sometimes also IL4 and IL9) and responding to tissue-damage signals such as IL33, TSLP, IL25.[205,206] In airway tissues, such as lung or nasal mucosa, ILC2s are able to migrate into tissues, usually responding to cytokines (IL25) or prostaglandins (PGD_2), inducing tissue eosinophilia ad mast cell response.[207] Interestingly, NK cells can inhibit the type 2-immune response of ILC2s, in a IFN-γ mediated way.[208] The modulation of peripheral allergy, occurring in tissues facing at the external environment, should be orchestrated by basophils and ILC2s, rather than single mast cells.

In lung and mucosal tissues of the upper airway tracts, ILC2s might have a strategic role for the ability of basophils to modulate allergy and participate in AIT.[209-211] This may represent an interesting issue for immunotherapy of airway allergens.[212] AIT is able to immuno-modulate ILC2s. Innate lymphoid cells can be grouped in at least three subgroups.[213] ILC2s are induced to produce type 2 cytokines, such as IL4, IL5, IL13 and IL9 by IL33, IL25 or TSLP.[214,215] In humans, the Lin^- $CD25^+$ $ST2^+$ c-Kit^+ $CD127^+$ $ICOS^+$ subpopulation of ILC2s, non expressing ILC3, CD4, NKp46, and RORγt, has been recently described.[215] The involvement of ILC2s in skin and airway allergy, such as allergic rhinitis, has been extensively studied in recent years.[216] A subcutaneous immunotherapy (SCIT) with airway allergens, such as grass pollen, reduced drastically the increase in ILC2s observed during seasonal allergy and identified as $CD117^+$ILC2s and IL-13^+ILC2s, though a short four-months SLIT did not get the same efficacy.[217] Actually, levels of circulating ILC2s appeared comparable in atopic and non atopic (non allergic) subjects, despite the differential regulation of these cells during allergy.[212 218]

Actually, basophil are able to regulate, via the IL4/IL4R-mediated signaling, many innate and type 2 immune cells, including ILC2s.[219] In this perspective, it is tempting to speculate that dermal ILC2s might even have a role in EPIT via either an IL25 or a TSLP-mediated pathway, with the major contribution of basophils. The role of IL25 and IL33 in basophil activation has been deepened quite recently in a human model of asthma. Following allergen inhalation challenge in asthmatic patients, an increase in circulating basophils expressing IL17RB, intracellular IL25 and the membrane marker ST2 was observed, and it was associated with a increase in type 2 cytokine-producing activated basophils when these cells were triggered with IL25 or IL33 *in vitro*.[220] This perspective assesses the "alarmin" role exerted by IL25 and IL33, also in basophils, not only in IL2Cs. Therefore, basophils actively participate in promoting an early pro-inflammatory and type 2 immune response in tissues triggered with allergens, particularly because IL25 and IL33 increase the migration potential of basophils towards damaged or inflamed tissues.[220]

Moreover, most probably basophils also orchestrate the modulation of allergy via their complex cross-talk with anti-inflammatory cells, including DCs, Tregs, Tr1, Th1 cells, IL10-producing $CD8^+$ T cells and others. This modulatory role might not be fully accomplished by IL33-triggered IL2cs, as their IL4 production promotes food allergy by blocking Treg function.[221] IL33 induce the production of IL4 also in activated basophils,[220] yet IL4 from basophils induces naïve $CD4^+$ T cells to differentiate in iTregs.[222] In this context, the production of IL9 from basophils should prevent the massive release of IL4 from Th2 cells, which could block the differentiation of iTregs by inhibiting the TGF-β mediated expression of Foxp3.[223]

Furthermore, IL33 induces the production of IL9 in basophils, besides $CD4^+$ T cells.[224] IL9 is predominantly produced by Th17 cells; basophils therefore collaborate with Th17 cells, which are induced by basophils themselves, in the release of IL9. This cytokine is fundamental to synergize with TGF-β in

the differentiation of Th17 cells from naïve $CD4^+$ T cells and also to enhance the suppressive activity of $CD4^+CD25^+Foxp3^+$ T regs, via a STAT3 and STAT5 signaling.[225]

Figure 2 summarizes this point.

IL2cs have been recently described as fundamental cells in allergy.[226,227] While IL9 is able to promote the production of TNF-α and IL6, in mast cells and basophils,[226] basophils should also be able to directly inhibit IL2Cs, most probably via an IL6-mediated signaling. This suggestion arises from the evidence that dendritic cells (DCs)-mediate innate and adaptive responses, during oral immunotherapy, either SLIT or OIT, and are modulated by a TLR-dependent mechanism and IL6. During SLIT, but not OIT, the TLR-induced production of IL6 from myeloid DCs (mDCs) is inhibited and upon OIT it affects also the production of IL10 in mDCs.[228] Moreover, OIT decreases IL6 production in plasmacytoid DCs (pDCs), while increases IFN-α expression,[228] which consequently inhibits the induction of IL13 release from pDCs; furthermore, both SLIT and OIT decreased the TLR-mediated Th2-type cytokine production from pDCs.[228]

In this context, basophils should act primarily as immunomodulatory cells, rather than effector pro-allergic leukocytes. They can either promote or dampen a pro-inflammatory response from an allergen challenge. As a matter of fact, basophils express FcδRs for IgD, which might represent an ancient form of immune surveillance.[229-231] Most probably, as emerging from some recent evidence, the interaction FcδR/IgD, which activates signal transduction in T cells,[232] in basophils may enhance the production and release of IL6.[233] IgD signal for basophils might be specific to drive these cells to a pro-inflammatory response. When serum IgDs level is elevated, as occurring in many autoimmune diseases, these immunoglobulins are potent inducers of the proliferation of peripheral blood mononuclear cells (PMNCs) and enhancers of the production of pro-inflammatory cytokines from these leukocytes, such as IL-1α, IL-1β, TNF-α, IL-6.[234] IgDs in serum induce also the proliferation of activated B cell subsets (i.e. $CD19^+CD23^+$, $CD19^+CD21^+$, $CD19^-CD138^+$ and $CD19^+IgD^+$) and also T cell subsets ($CD4^+CD154^+$ and $CD4^+CD69^+$).[234]

Interestingly, past evidence suggested a role for IgDs in blocking B-cell apoptosis (so interfering with immune tolerance) following the antigen-mediated cross-linking of membrane-bound IgM (sIgM).[235] Whether a role for IgDs in tolerance induction and immuno-suppression should exist and if this role may affect the modulatory role of basophils in the relationship with Tregs, is still a mater of debate. IgDs elicit the production of IL10 in PBMCs.[234] Immunoglobulin D should be a key regulator in the humoral immune response, particularly important in the function of the B cell receptor (BCR).[236,237] In this perspective, IL6 produced by IgD-activated basophils, might help B cells in producing antibodies and $CD4^+$ T helper cells in their activity by increasing IL21 production.[238]

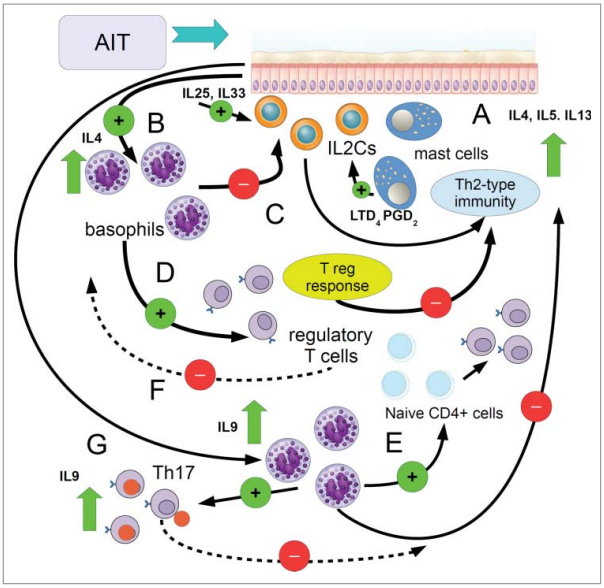

Figure 2. Cartoon showing the complex regulatory activity of basophils following AIT. A) Initially, allergen challenge elicits a mast cell response, which triggers the type2 innate lymphoid cells (IL2Cs), via mediators such as LTD_4 and PGD_2, to support a type 2 immune response, with the production of IL4, IL5, IL13. B) Tissue allergen challenge induces the production of alarmins and cytokines, such as IL25 and IL33, which induce IL2Cs to the Th2 response and activate basophils to produce IL4, which in turn inhibit the activity of IL2Cs, to reduce (C) the onset of inflammation; D) In these circumstances, IL4 from basophils induces the differentiation and activation of Tregs, which in turn dampen allergy reactions (E) through cytokines (IL10, TGF-β) and IgG_4; F) here basophils produce also IL9, which helps the differentiation of Tregs from naive$CD4^+$ T cells; G) the promotion of the development of IL17-producing T regs (Th17) from basophils contributes both in increasing IL9 and inhibiting CD4+CD25+Foxp3+ T regs differentiation, in order to switch off the Treg-mediated signaling. Green circle: activation (+), red circles inhibition (−).

Conclusions

The activity of basophils in the complex network of the immune system has gaining major attention in recent years, due to the many fundamental insightful results about their modulatory rather than effector actions in allergy and immunotherapy.

Recently, new advances have been proposed in targeting basophils or mast cells, via their membrane receptors or released mediators, such as serine proteinases, histamine 4-receptor, 5-lipoxygenase-activating protein, 15-lipoxygenase-1, prostaglandin D2, and proinflammatory cytokines, giving the opportunity to plan possible promising therapeutic suggestion to dampem allergy inflammtion and the role of these cells in exacerbating immune disorders.[239]

The many relationships of basophils with both innate ad acquired immunity suggest for the existence of a tuning role in balancing host's response to external stimuli or allergens, which basophils should exert by interacting with immune cells in lymphoid tissues and in epithelia and sensing soluble mediators, such as immuno-globulins, cytokines, vaso-active amines, leukotrienes and prostaglandins, via membrane receptors, which could be used also to gate basophils in cellular diagnostic assays. The central regulatory role in the immune system, which these leukocytes are endowed with, makes basophil a fundamental target for immunotherapy and a fascinating topic for further research in medical immunology.

Acronyms

C/EBPα	CCAAT-enhancer binding protein alpha
CCR1	C-C chemokine receptor 1
COX	cyclooxygenase
CTLA-4	Cytotoxic T-Lymphocyte Antigen 4

FOG-1	friend of GATA 1
GATA	(consenss seqence guanine-adenine-thymine-adenine)
GARP	glycoprotein A repetitions predominant
ICOS	Inducible T-cell costimulator
IFN	interferon
IL	interleukin
IRF-8	interferon regulatory factor-8
LAP	latency-associated peptide
LTB4	leukotriene B4
mMCP-8	mouse mast cell protease 8
MCP-1	monocyte chemotactic protein-1
MITF	microphtalmia-associated transcription factor
OT	obese transgenic
PD-1	programmed cell death 1
PGE2	prostaglandin E2
P1RUNX	Promoter 1 Runt-related transcription factor 1
RANTES	regulated on activation, normal T cell expressed and secreted
RORγr	RAR-related orphan recetor gamma t
SHIP-1	SH-2 containing inositol 5′ polyphosphatase 1
STAT5	signal transducer and activator of transcripton 5
TGF	tumor growth factor
TLR	toll-like receptor
TSLP	thymic stromal lymphopoietin
TNF-α	tumor necrosis factor alpha

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

A special thank to Dr. James Walmadge, Senjor English Lectuter, University of Padua, for his precious help in the manuscript revision for English grammar and syntax.

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