Basophil Activation Antigens: Molecular Mechanisms and Clinical Implications

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Abstract: Basophil activation is a key finding in allergic reactions and also observed quite frequently in infectious diseases and autoimmune disorders. In allergic reactions, basophil-derived mediators such as histamine, contribute essentially to clinical symptoms. During IgE-dependent degranulation of basophils, a number of cell surface membrane and cytoplasmic molecules become activated, show altered expression, or are translocated into the cell surface. Although little is known so far about the exact role of these activation-linked cell surface antigens, several of them are employed as diagnostic parameters in allergic disorders. Other molecules are involved in the process of signalling and the consecutive release of pro-allergic mediators, and have therefore been proposed as potential targets of therapy. The current article provides a summary on activation-linked cell surface and cytoplasmic antigens in basophils, with special reference to potential mechanisms underlying re-translocation or over-expression in activated cells, relevant signalling pathways, and clinical implications.

INTRODUCTION

Blood basophils are unique effector cells of the immune system. These cells contribute essentially to allergic and inflammatory reactions [1-4]. Unlike mast cells, basophils are circulating cells and originate from granulocytic progenitors [5-7]. Basophils also differ from mast cells in cytokine receptor expression, response to various interleukins (ILs) and tissue hormones, and expression of chymotryptic enzymes and proteoglycans (Table **1**) [8-12]. Nevertheless, basophils and mast cells share several important features, including expression of the IgE receptor, production and storage of histamine and other proinflammatory mediators, and expression of certain cell surface antigens including CD9, CD33, CD45, and CD63 (Table **1**).

In common with all other circulating leukocytes, basophils originate from immature uncommitted CD34+ bone marrow progenitor cells [5-7]. A number of different cytokines and other factors are involved in the regulation of growth, differentiation, and maturation of lineage-committed and multipotent basophil precursor cells. The most important growth factor for basophils appears to be IL-3 [13-15]. Other cytokines contributing to basophilopoiesis are IL-5, granulocyte macrophage colony-stimulating factor (GM-CSF), and nerve growth factor (NGF) [15-17]. These cytokines act on immature and mature basophils through specific receptors [18-20]. In fact, these regulators not only trigger differentiation and maturation of basophils but also the function of ma-

ture blood basophils, including survival, adhesion, chemotaxis, releasability, and cytokine production [21-27]. Mature basophils also express high affinity receptors for IgE, which play an essential role in allergic diseases [28-30]. Notably, during an allergic reaction, IgE receptors are cross-linked on basophils by an allergen via specific IgE, resulting in degranulation and mediator release, and thus in specific symptoms in allergic reactions [28-30].

During the past few decades, our knowledge on basophil activation through IgE-dependent reactions or cytokine mediation has increased considerably [28-30]. Moreover, a number of additional cell surface antigens involved in basophil activation have been identified [31-40]. The current article provides a summary of our current knowledge on cell surface activation-linked antigens on human basophils, with special focus on biochemical mechanisms underlying expression and activation of these antigens, and potential clinical implications.

CELL SURFACE PHENOTYPE OF RESTING BLOOD BASOPHILS

Resting blood basophils express a unique composition of cell surface antigens, including the high-affinity receptor for IgE, receptors for various interleukins such as IL-3 and GM-CSF, chemokine receptors, various complement receptors including CR1 (CD35) and the C5a receptor (CD88), several adhesion molecules such as beta 1 and beta 2 integrins, or ICAM-1 (CD54), and various other myeloid cell surface antigens [9-12,18-20,33,41-45] (Table **1**). In common with all leukocytes, basophils display the pan leukocyte antigen CD45 and the hyaluronan receptor CD44 [9-11] (Table **1**). Unlike mast cells, resting blood basophils do not express

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**Table 1. Differentiation Antigens Expressed in Basophils and Mast Cells**

Antigen	Expressed in		Function and/or Clinical Impact
	Basophils	**Mast Cells**	
Surface			
IgER/FcεRI	+	+	mediator release
IL-2R/CD25	+	-(+)*	unknown
IL-3R/CD123-CD132	+	-	multiple (basophils)
IL-4R/CD124	+	+	unknown
Siglec-3/CD33	+	+	target (neoplastic cells)
CR1/CD35	+	-	complement activation
Pgp-1/CD44	+	+	hyaluronan receptor
CLA/CD45	+	+	pan leukocyte marker
ICAM-1/CD54	+	+	adhesion
C5aR/CD88	+	+/-	chemotaxis, release
SCFR/KIT/CD117	-(+)**	+	multiple (mast cells)
IL-8RA/CD128	+	-	chemotaxis
IL-18R	+	-	unknown
Mediators			
Histamine	+	+	mediator of allergy
Heparin	-	+	ATIII and tPA co-factor
Tryptase	+/-(+)**	+	protease, IHC marker
Chymase	-	+	protease, IHC marker
Activation Antigens			
AP-N/CD13	+	+/-	upregulated (IgE+allergen)
LAMP-3/CD63	+	+	classical baso test
LAMP-1/CD107a	+	+/-	upregulated (IgE+allergen)
LAMP-2/CD107b	+	+/-	upregulated (IgE+allergen)
Endolyn/CD164	+/-	-	upregulated (IgE+allergen)
E-NPP3/CD203c	+	+/-	upregulated by IgE+ allergen or IL-3 (basophils)
Other Granule Antigens			
Basogranulin/BB1	+	-	IHC marker (basophils)
2D7	+	-	IHC marker (basophils)

*In systemic mastocytosis, neoplastic mast cells aberrantly express CD25. **Immature basophils express low amounts of KIT and tryptase. SCF, stem cell factor; IHC, immunohistochemistry; AP-N, aminopeptidase-N.

substantial amounts of KIT (CD117) or vitronectin receptor (CD51/CD61) [9-11]. However, immature basophil progenitor cells may express low amounts of KIT, and the same may hold true for rapidly mobilized (activated) blood basophils [46-48].

A unique and rather specific marker for basophils and their progenitor cells is the ecto-enzyme ectonucleotide pyrophosphatase/phosphodiesterases 3 (ENPP-3) clustered as CD203c [33,37-40]. In fact, CD203c is expressed on immature basophil-committed CD34+ progenitor cells, immature bone marrow basophils, and mature blood basophils [33]. As will be discussed below, CD203c is also an activation-linked cell surface antigen on human basophils. Other blood leukocytes do not express substantial amounts of CD203c. However, tissue mast cells express low levels of CD203c, and when activated or transformed (neoplastic mast cells), the levels of CD203c on mast cells increase substantially [49]. Other cell surface antigens that have been described as activation-linked markers and are detectable on resting blood basophils include aminopeptidase N (CD13), the lysosomal membrane antigens LAMP-1 (CD107a), LAMP-2 (CD107b), and LAMP-3 (CD63), and endolyn (CD164) [37-40,50-53]. Most of these antigens are not only detectable on
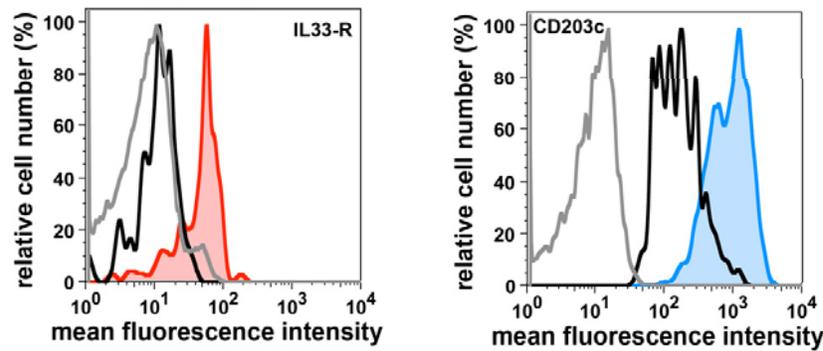
Fig. (1). Effects of IL-3 on expression of IL33-R and CD203c on human basophils.

Peripheral blood basophils obtained from a healthy donor (left panel) and from a patient with chronic myeloid leukemia (right panel) were incubated with control medium (black line, open histogram) or recombinant interleukin-3, IL-3 (100 ng/ml) (grey histogram) at 37°C for 15 minutes. Then, basophils were stained with an antibody against the IL-33 receptor ST2 and an antibody against CD203c, and were analyzed by multicolor flow cytometry. Incubation with IL-3 resulted in an enhanced (de novo) expression of the IL-33R (ST2) in normal basophils (left panel) and CML basophils (not shown), and in an enhanced expression of CD203c on normal basophils (not shown) and CML basophils (right panel). The grey line (open histogram) shows the isotype-matched control antibody.

the surface of basophils but also in cytoplasmic (lysosomal) membranes within basophils.

CONSEQUENCES OF IgE RECEPTOR CROSS-LINKING

During IgE receptor cross-linking, a number of signal transduction events and biochemical processes are initiated that lead to basophil activation and consecutive degranulation [2,8,10,28,29]. Signalling molecules that are involved in IgE receptor activation and downstream signalling cascades in basophils (and mast cells) include (among others) Fyn, Syk, and Lyn, the PI3-kinase, Akt, and phospholipase C, Ras, Raf and the MAP kinases, as well as Src, Btk, Jnk, and PKC [2,8,10,28-30,54-57]. In addition, Jak2 and Stat5 activation may be initiated after IgE receptor cross-linking in basophils [54-57]. Most of these signalling pathways are interconnected and are considered to act together to lead to basophil activation with consecutive degranulation and mediator release as well as cytokine production, cytokine release, and generation of lipid mediators in basophils [2,8,10,28-30,54-57]. Moreover, upon IgE-receptor cross linking in basophils, a number of cell surface membrane antigens appear to be translocated from cytoplasmic (lysosomal) membranes onto the cell surface [37-40,50-53]. These upregulated cell surface membrane molecules include (among others) CD11b, CD13, CD63, CD107a, CD107b, and CD203c. IgE-mediated upregulation of these antigens may depend on distinct signalling pathways including the PI3 kinase/Akt pathway [39,40,50,58].

EFFECTS OF INTERLEUKIN-3 AND OTHER INTERLEUKINS

Interleukin-3 (IL-3) not only promotes the differentiation and survival of human basophils but also releasability, adhesion, migration, cytokine production, and surface receptor expression [15,18-24,59-61]. In allergic patients, IL-3 may even induce mediator secretion in the absence of other agonists (i.e. allergens) [22]. An important aspect is that IL-3 markedly triggers the expression of other cell surface antigens on human basophils. Such upregulated antigens include CD203c and the receptor for IL-33 (ST2) [62,63]. The no-

tion that IL-3 promotes the expression of CD203c has to be taken into account when using CD203c as a basophil activation marker in allergic reactions [40,64]. An interesting aspect is that ST2 (IL-33 receptor) is not detectable on resting normal basophils by conventional flow cytometry but is detectable on IL-3-exposed (primed) basophils (Fig. **1**). In line with this observation, IL-33 synergizes with IL-3 in promoting basophil activation and mediator secretion [63]. Similar to IL-3, IL-5 and GM-CSF can also activate human blood basophils and promote their releasability [15,19,23-25]. In addition, the receptors for IL-3, IL-5, and GM-CSF on basophils share a common signal-transducing beta-chain [19,20]. However, despite expression of a common signalling receptor-chain, not all effects of IL-3 on basophils are mimicked by IL-5 and GM-CSF, or are less pronounced upon exposure to GM-CSF or IL-5 compared to effects seen with IL-3. Other cytokines and interleukins such as nerve growth factor (NGF) or IL-33 also trigger the activation of resting human blood basophils [17,63]. However, again, the effects of these cytokines usually are less pronounced compared to effects provoked by IL-3 [65]. All in all, IL-3 appears to be a most effective cytokine-agonist for human blood basophils.

Following cytokine exposure, a complex network of signal transduction molecules and pathways are activated in basophils. Key signalling molecules contributing to basophil activation after exposure to IL-3 are similar or the same compared to that involved in IgE receptor downstream signalling, and include the PI3-kinase/Akt/mTOR pathway, the MEK/ERK pathway, and the JAK/STAT pathway. An interesting aspect is that signalling through the IL-3 receptor and IgE receptor share many similarities [66,67]. Moreover, it has been reported that the FcR gamma-chain, which can serve as component of the IL-3 receptor, is required for IL-3-induced production of IL-4 in mouse basophils [45]. Whether this holds also true for human blood basophils remains at present unknown.

Recently, the effects of various novel cytokines on signal transduction in human basophils have been explored and compared to IgE-dependent and IL-3-induced activation. Interestingly, the signalling pathways triggered by IL-33 in

blood basophils are different from that triggered by IL-3. Whereas IL-3 primarily activates the JAK/STAT pathway and ERK-activation in human basophils, IL-33 was found to activate the NFkB and p38 MAP-kinase pathway [63]. In line with this observation, IL-33 did not mimic all effects of IL-3 on human basophils. Likewise, in contrat to IL-3, IL-33 did not prime human basophils for C5a-induced LTC4 generation [63]. Furthermore, unlike IL-3, IL-33 does not promote the expression of CD203c or other surface molecules in human basophils (unpublished observation). This is of interest as IL-33 has been described to trigger releasability in human basophils [63,68,69]. All these observations suggest that translocation of membrane antigens onto the cell surface is not invariably linked to (not sufficient for) mediator secretion in basophils.

EFFECTS OF OTHER NATURAL LIGANDS

Apart from IL-3 and other interleukins, a number of additional natural ligands can promote basophil activation. Among these natural regulators are the interferons, the complement products C5a and C3a, and various chemokines such as MCP-1 or IL-8 [2,3,23-25,41-44,70-77]. Whereas interferon-alpha and interferon-gamma promote basophil releasability after exposure for 12-24 hours (presumably via effects mediated by accessory cells) [70,71], C5a and the chemokines rapidly induce mediator secretion as well as chemotaxis in basophils [41,44,72,73]. There are a number of other effects these natural ligands have on basophils, including the regulation of cytokine production and release, survival, and adhesion [75-77]. Together, a number of different cytokines and chemokines regulate basophil function relevant to allergic or other inflammatory reactions. It is assumed that these ligands act together to trigger basophil activation. Moreover, a number of additional intrinsic and extrinsic factors, including the underlying disease, genetic background, micro-environment, presence and type of microbes, and drug intake may play a role and may determine releasability in human blood basophils [78-81].

BASOPHIL ACTIVATION ASSAYS

A number of different assays for measuring basophil activation have been proposed in the past. The first robust assay based on IgE-dependent upregulation of a cell surface antigen on basophils was the CD63-test, also known as basophil-activation-test or ´baso-test´ [31,32,34,52,53,82,83]. Although exhibiting several limitations, the assay is employed as a standard in various centers. One limitation is that CD63 is not specific for basophils and is a less sensitive activation parameter. An alternative assay that exhibits high sensitivity and specificity is based on IgE-dependent upregulation of CD203c [36-40,64,84,85]. The advantage of this assay is that CD203c is specific for blood basophils (not found on other blood leukocytes) and that CD203c is a sensitive activation parameter [36-40]. On the other hand, CD63 may be more specific for allergic (IgE-dependent) reactions and may be less susceptible to non-specific upregulation by cytokines or other factors [36-39,62]. However, CD63 is not specific for basophils and may sometimes show false negative results because of its relatively low sensitivity. As a consequence, we recommend that basophil activation is measured by employing both CD63 and CD203c in a combined approach by multi-color flow cytometry [38,64]. An-

other important aspect is that not only the percentage of reactive basophils should be counted, but also the mean fluorescence intensity (MFI) [38,64]. In fact, using a standardized approach measuring the MFI of the test marker against the MFI of the isotype control should yield reliable and reproducible results for allergen-induced basophil activation [38,64]. Finally, it is important to select the optimal set and type of allergen(s) to explore the allergic status at the effector cell level. Today, the use of recombinant allergens is recommended in basophil activation assays whenever possible [38,64].

SIGNALLING AND EFFECTOR MOLECULES AS POTENTIAL TARGETS OF THERAPY

A number of signalling molecules and downstream effector molecules are critical to basophil activation and the consecutive release of pro-inflammatory mediator substances [2,8,10,28-30,54-57]. Several of these signalling molecules have recently been discussed as potential targets of therapy in allergic disorders [86-90]. A list of potential targets and some targeted drugs are shown in Table **2**. In fact, there are a number of targeted drugs recognizing critical kinases and other targeted drugs used in clinical trials to treat cancer patients or patients with severe autoimmune disorders [91-96]. However, most of these drugs also have significant side effects, especially when multiple targets are identified [91-96]. Notably, most signal transduction inhibitors are not specific drugs, but are broadly acting drugs recognizing a number of different target kinases in various effector cells [97-99]. As a result, most kinase-targeting drugs cannot be used in patients with allergic disorders. Good examples for multi-kinase inhibitors that are applied in cancer patients and block IgE-dependent histamine release by interfering with signal transduction pathways in basophils are dasatinib and midostaurin (PKC412) [100,101]. Many more kinase blockers are available and applied in cancer patients, but their effects on basophils or mast cells have not been investigated yet. More specific kinase inhibitors have also been developed, and some of them show inhibitory effects on basophil activation [102-111]. Several of these inhibitors interact with and block Syk, suggesting that this kinase may play a particular role as potential drug target in basophils. Several of the above targets may also be involved on the translocation of activation-linked cell surface antigens (including CD63 and CD203c) on basophils. Indeed, basophils may be less capable of upregulating activation-linked cell surface antigens and to release proinflammatory mediators during treatment with multikinase inhibitors [112]. Whether such drug effects on IgE-mediated upregulation of CD antigens can be employed to monitor drug effects (on basophils) in these patients remains at present unkown.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Activation-linked cell surface membrane antigens on basophils are increasingly used in research and in practice, in order to explore the biology of basophils, their role in various pathologic reactions, and to determine their responses to allergens in allergic disorders. Moreover, basophil activation antigens are increasingly employed to screen for drug effects and to determine disease activity and responses to immunotherapy and other drugs in patients with allergic diseases.

Table 2. Examples for Kinase-Targeting Drugs and their Effects on Basophils

Targeted Drug	Major Target(s)	Blocks IgE-Mediated Basophil Activation
Broadly Acting Drugs		
PKC412 (midostaurin)	PKC, KDR, KIT, FLT3, ….	+
Dasatinib	Abl, KIT, Lyn, Btk, Fyn, Src, …	+(-)*
AMN107 (nilotinib)	Abl, KIT, PDGFR, …	-
STI571 (imatinib)	Abl, KIT, PDGFR, …	-
Piceatannol**	Syk, STAT5, ZAP70, …	+
Shikonin	Syk, Lyn, …	+
More Specific Drugs		
BAY 61-3606	Syk	+
R406	Syk	n.k.
R112	Syk	n.k.
NVP-BEZ235	PI3-K & mTOR	+/-
Rapamycin	mTOR	-
Cyclosporin-A	Calcineurin	+
FK506	FK-BP	+

mTOR, mammalian target of rapamycin; n.k., not known; FK-BP, FK506-binding protein. *At low dose, dasatinib sometimes even promotes IgE-dependent histamine release in basophils, whereas at higher concentrations (>100 nM), dasatinib completely blocks IgE-dependent histamine release in all donors [100]. **Piceatannol is an experimental compound but not used in clinical trials.

During the past few years basophil activation antigens have been linked to certain signalling pathways and signalling molecules relevant to degranulation and mediator release. Several of these signalling molecules may represent potential drug targets. There is hope for the future that basophil research will employ these basophil-targets and basophil-activation markers with the goal to improve diagnostic assays as well as therapy in allergic patients.

ACKNOWLEDGEMENT

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