



Basophil and mast cell activation tests by flow cytometry in immediate drug hypersensitivity: Diagnosis and beyond

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ABSTRACT

Immediate drug hypersensitivity reactions (IDHRs) constitute a significant health issue with serious consequences of diagnostic error. The primary diagnostics to document IDHRs usually consists of quantification of drug-specific IgE (sIgE) antibodies and skin tests. Unfortunately, the positive predictive value (PPV) and negative predictive value (NPV) of these tests are not absolutely, which leaves room for new tests. Over the last two decades, the basophil activation test (BAT), in which ex vivo activation of individual basophils is quantified by flow cytometry, has emerged as a reliable complementary diagnostic to document IDHRs, to explore allergenic recognition, to study cross-reactivity and to monitor therapy. However, the BAT is technically challenging requiring specialized personnel and equipment, fresh samples and the technique is lost as a diagnostic in patients showing a non-responder status of their cells. By consequence, the BAT has still not entered mainstream application. In contrast, mast cell activation tests (MATs) use serum samples that can be frozen, stored, and shipped to a recognized reference centre experienced in mast cell (MC) lines and/or cultures and capable of offering batch testing with necessary quality controls. This review does not only highlight the use of the BAT and MAT as diagnostics in IDHRs, but also outlines the potential of both techniques in further exploring and unveiling the mechanisms that govern drug-induced basophil and MC activation and degranulation.

1. Introduction

Immediate drug hypersensitivity reactions (IDHRs) constitute a significant health issue with serious consequences and financial burden of mis- and overdiagnosis. However, correct diagnosis of IDHRs can pose a significant challenge, mainly because of unknowns in the mechanistic processes that govern basophil and/or mast cell (MC) degranulation and uncertainties associated with the available in vitro and in vivo diagnostics. Activation of basophils and MCs via cross-linking of antigen-specific IgE (sIgE) antibodies bound to the high-affinity receptors (FcεRI) in the cell surface is the hallmark of classical Gell and Coombs type I hypersensitivity reactions (Gell and Coombs, 1963). Typical examples of such sIgE/FcεRI-dependent reactions are IDHRs to β-lactam

antibiotics (penicillins, cephalosporins, monobactams) and the biguanide antiseptic chlorhexidine (Romano et al., 2020; Rose et al., 2019). However, since 2015 (McNeil et al., 2015), evidence, mainly obtained from rodent models and in vitro studies, has been mounting that the MRGPRX2 pathway heralds a new area in our understanding of sIgE/FcεRI-independent IDHRs (Porebski et al., 2018). This pathway seems to be involved in reactions towards fluoroquinolones (FQs), opiates and the bradykinin receptor 2 antagonist icatibant (Porebski et al., 2018; Van Gasse et al., 2017a; Van Gasse et al., 2017b). The situation seems more complex for curarizing neuromuscular blocking agents (NMBAs), which can trigger anaphylaxis via both clinically indistinguishable pathways (Porebski et al., 2018; Van Gasse et al., 2019; Ebo et al., 2020a). However, to date, clinical evidence for MRGPRX2-dependent IDHRs is scarce

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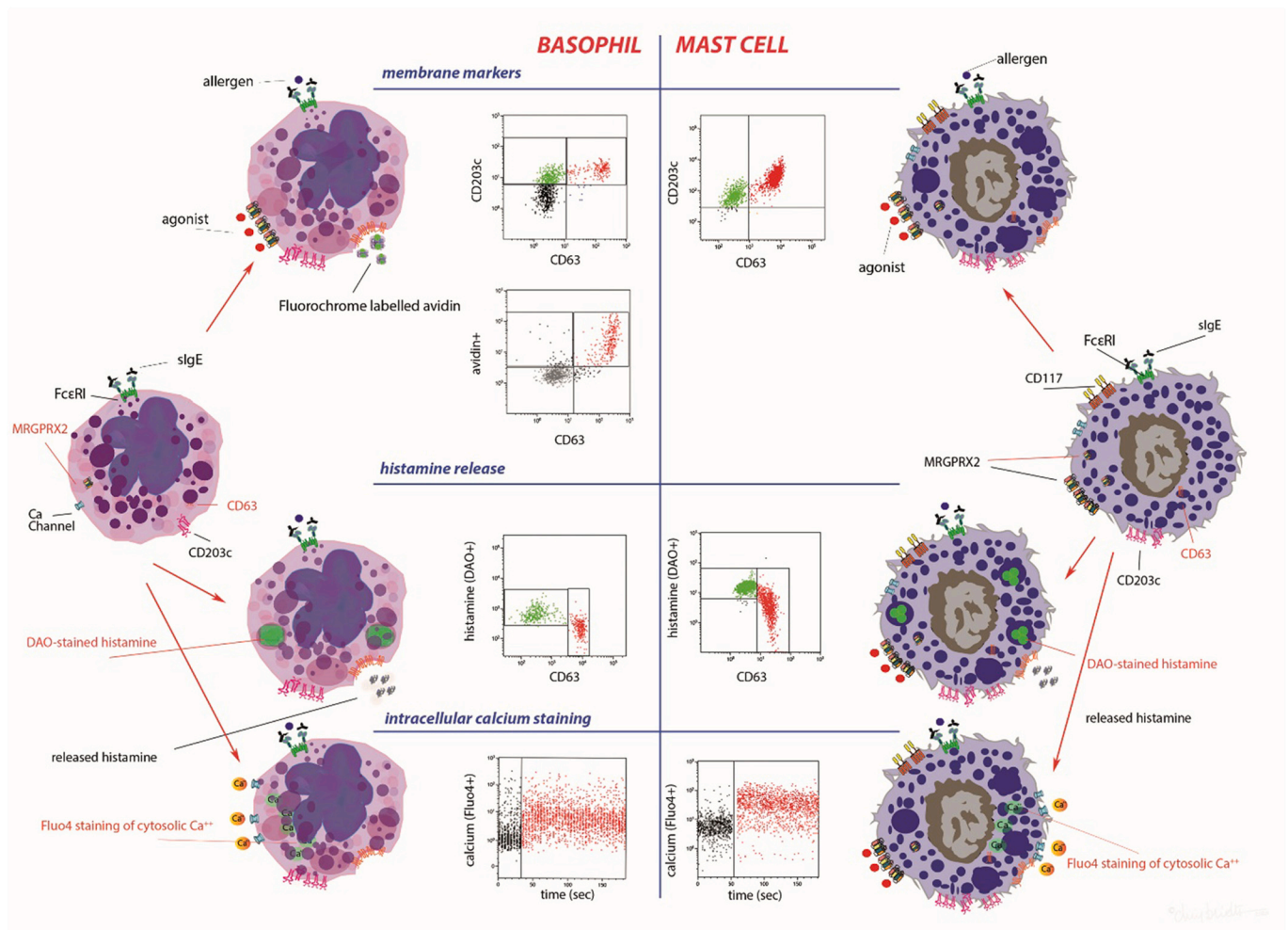


Fig. 1. Basophils (left) and mast cells (right) express various molecules that can be used in isolation or in combination to identify the cells and measure their activation/degranulation status by flow cytometry in a technique designated as basophil/mast cell activation test (BAT/MAT). Basophil and mast cell activation/degranulation can occur via IgE/FcεRI-dependent and IgE/FcεRI-independent pathways (e.g. by activation of the MRGPRX2 receptor). Individual cell activation/degranulation can be measured flow cytometrically on 4 levels. First, via appearance or up-regulation of surface markers (such as CD203c, CD63, and avidin binding of membrane-associated exteriorized anionic proteoglycans released from granules). Second, via phosphorylation of signaling molecules (such as p38MAPK and STAT5, not shown). Third, via changes in mediator content (such as decrease of histamine or increase of trapped cytokines or their mRNA). Fourth, via increased intracellular calcium staining. Intracellular molecules are denoted in pink. For more details and references on the identification and activation/degranulation markers see text. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and a method to unambiguously document such reactions is lacking (Ebo et al., 2020a). In a patient who experienced an IDHR, a negative sIgE result does not prove a MRGPRX2 reaction. Neither skin testing nor drug challenges can discriminate between reactions from sIgE/FcεRI cross-linking and nonspecific MRGPRX2 activation. As such, there is a need for additional test - or combinations thereof - that might further unveil the role and mechanisms of basophil and MC activation in IDHRs. Such tests might not only contribute to correct diagnosis but also advance and shift mechanistic paradigms and enhance the (re)classification of type I drug reactions.

Here, we aim at summarizing the current applications of ex vivo basophil and in vitro mast cell (MC) activation tests by flow cytometry (henceforth designated as BATs and MATs) in IDHRs, which clearly extend beyond mere diagnostic aids. We hypothesize on the potential of BATs and MATs to discriminate between the sIgE/FcεRI and the MRGPRX2 endotype of IDHRs, especially when the two tests are performed in parallel.

2. Basophils and mast cells

Basophils and MCs are granulocytes that develop from CD34⁺ve

pluripotent progenitor stem cells. Circulating basophils and tissue-resident MCs can be activated in various ways, which may be differentiated according to whether or not cross-linking of the membrane-embedded sIgE/FcεRI complexes is involved (Fig. 1). IgE/FcεRI-dependent activation and degranulation largely occurs via (glyco)protein allergens, chemical allergens, (mostly IgG isotype) autoantibodies directed against the FcεRI receptor or IgE bound to surface FcεRIs (Kalesnikoff and Galli, 2010), or by molecules such as lectins with a binding specificity that matches the glycosylation of IgE and/or FcεRI (Haas et al., 1999; Pramod et al., 2007; Krithika et al., 2018). sIgE/FcεRI-independent activation and degranulation mainly result from nonspecific occupation of receptors for endogenous (e.g. cytokines, anaphylatoxins, chemokines, IgG, neuropeptides) or exogenous (e.g. pathogen-associated molecular patterns) substances (Finkelman et al., 2016). Amongst these nonspecific receptors is the Mas-related G protein-coupled receptor X2 (MRGPRX2).

The Mas-related G protein-coupled receptors (MRGPRs) comprise a group of receptors named after the first discovered member, Mas (Young et al., 1986) and have been reviewed by Bader et al. (2014). Today, MRGPRs are divided into several subfamilies that have been renamed according to a novel nomenclature (<http://www.guidetopharmacology>).

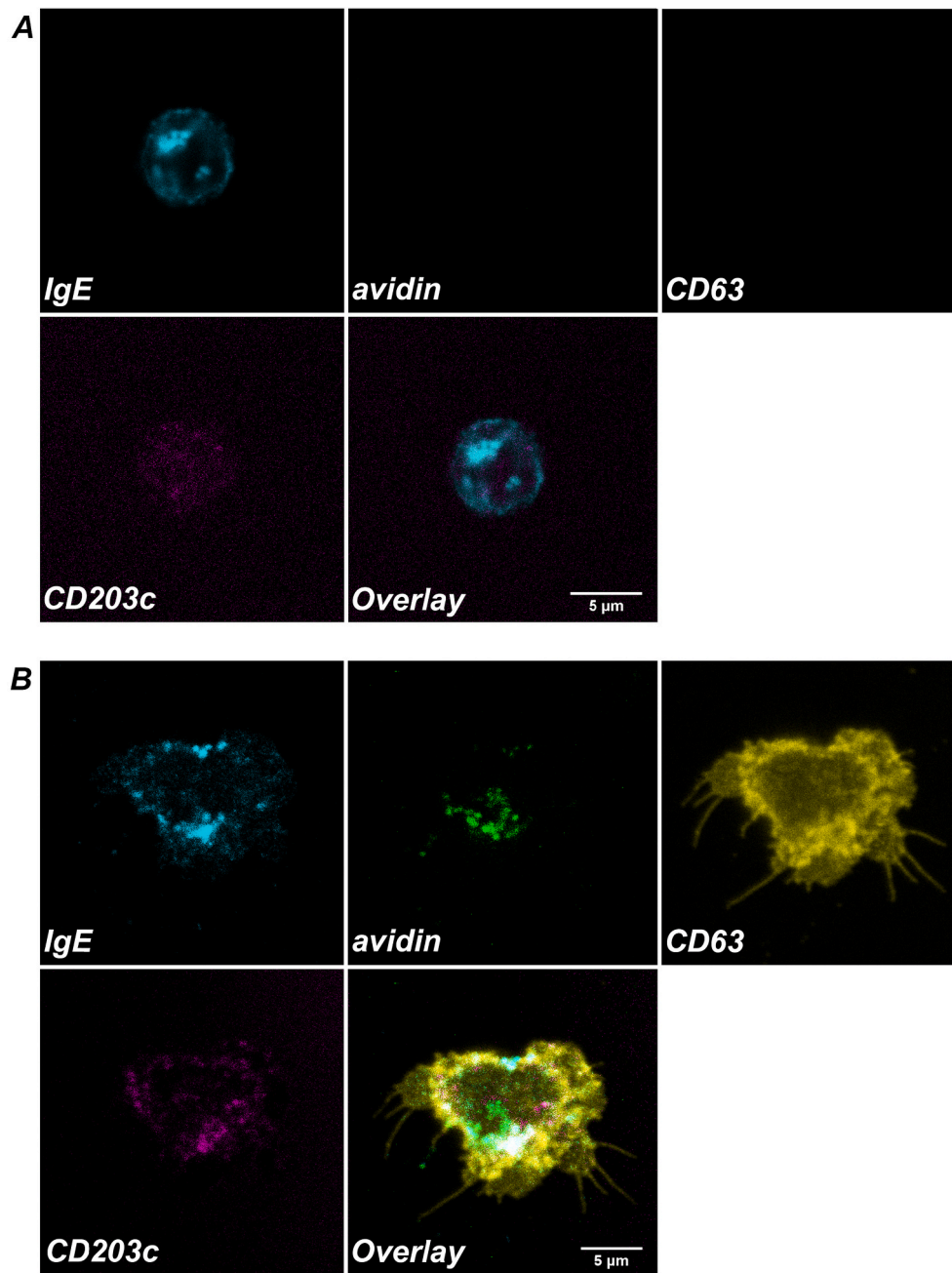


Fig. 2. Confocal images of (A) unstimulated basophil and (B) anti-IgE stimulated basophil stained for membrane IgE, avidin, CD63 and CD203c. On the stimulated basophil (B) there is appearance of CD63, binding of avidin and an increase of CD203c on the surface of the cell.

org) including the primate-specific MRGPRX subfamily. Human MRGPRX are expressed by several cell types including dorsal root ganglion neurons and tryptase- and chymase-containing connective tissue mast cells (MC_{TC}), as found in the skin (Varricchi et al., 2019). Moreover, quantitative PCR studies have shown that MCs from the skin show high expression of the receptor (Tatemoto et al., 2006). Whether MRGPRX2 is also constitutively expressed by human basophils, as claimed recently by Wedi et al. (2020a) is an ongoing subject of controversy (Sabato et al., 2017; Elst et al., 2020a; Wedi et al., 2020b; Sabato et al., 2020) and is addressed below. Its presence, or absence, will ultimately determine whether or not these cells will respond to MRGPRX2 agonists, including many drugs (Porebski et al., 2018).

3. Basophil and mast cell activation tests: general principles

3.1. Basophil activation tests (BATs)

For about half a century, ex vivo basophil activation studies have mainly focused on histamine and leukotriene release in supernatants of activated cells (Demoly et al. (2003)). Although these techniques for many remain the ‘reference test’ for effector cell activation and degranulation, mediator release assays have largely failed to enter mainstream application, due to the fact that they are time- and cost-consuming and require a two-step approach, i.e. cell isolation and incubation, and quantification of mediators.

The foundations of modern flow cytometric BATs date back from the early nineties, with the discovery of lysosomal-associated membrane protein 3 (LAMP3 or CD63) as a degranulation marker of basophils

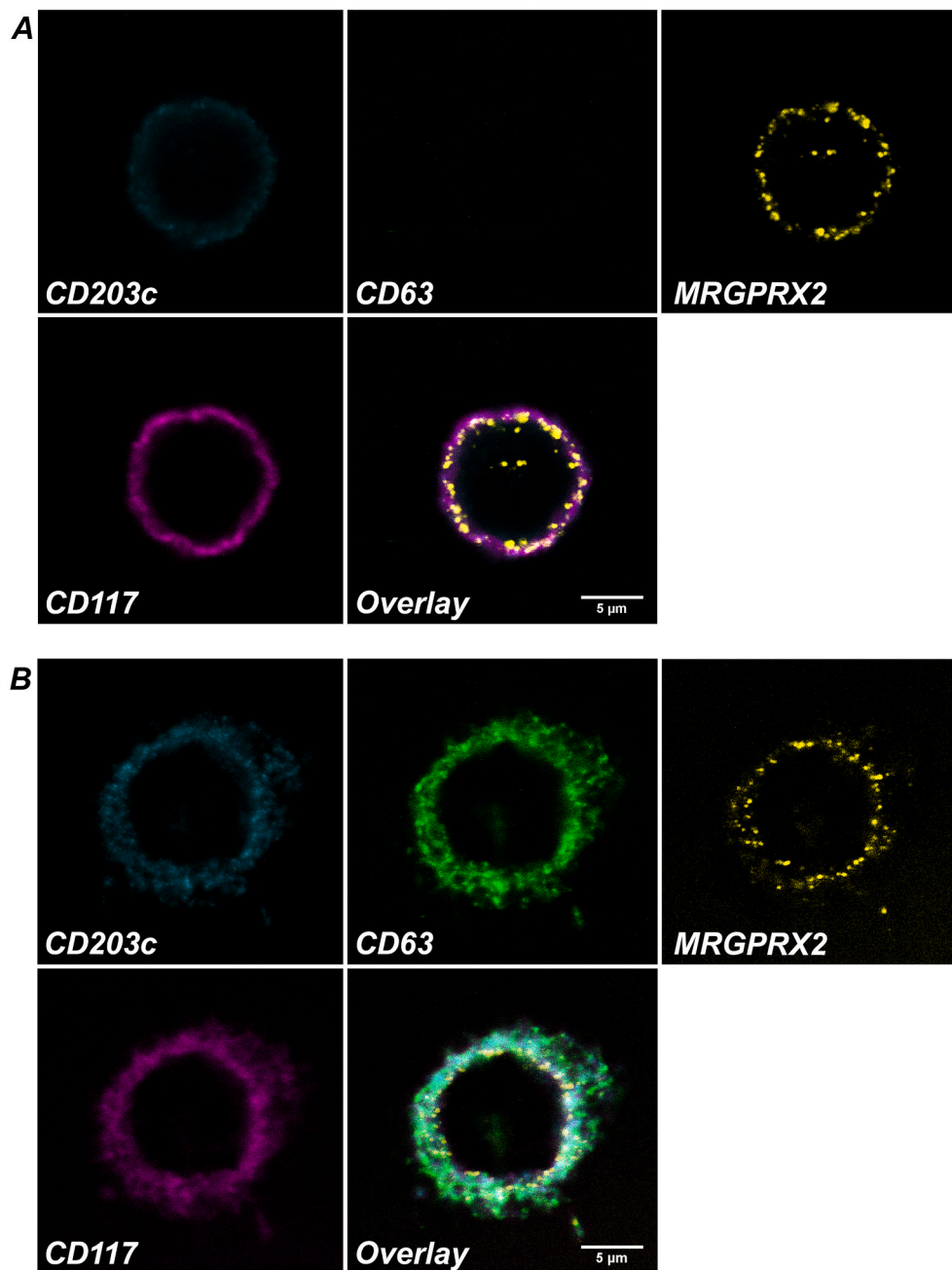


Fig. 3. Confocal images of (A) unstimulated mast cell and (B) substance P stimulated MCs stained for membrane CD203c, CD63, MRGPRX2 and CD117. On the stimulated mast cell (B) there is appearance of CD63 on the surface of the cell. The expression of the other markers is similar between unstimulated and stimulated conditions.

(Knol et al., 1991). Today, different protocols enabling detection of membrane alterations, intracellular changes and exteriorization of granular content have been developed. Traditional BATs use whole blood and rest on cellular identification and quantification of activation and/or degranulation markers on the membrane of individual cells. These changes are directly detectable and quantifiable at a single-cell level using specific fluorescent-conjugated monoclonal antibodies. In most studies, basophils have been identified by their scatter characteristics, reflecting cellular size and granularity together with staining of membrane markers such as IgE/CD203c, CCR3/CD3 or CD123/HLA-DR. Thereafter, after activation, the appearance or up-regulation of specific markers, such as CD203c or CD63, is quantified, with degranulating cells traditionally defined as being double-positive for CD203c and CD63. For a comprehensive description of kinetics, cellular localization,

signalosome, different relationship to different forms of degranulation of these activation markers and other means to study ex vivo basophil stimulation, the reader is referred elsewhere (MacGlashan Jr., 2013; Ebo et al., 2020b). A detailed description of our BAT protocols can be found in (Elst et al., 2020b; Bridts et al., 2020; Ebo et al., 2020c). Today, as shown in Figs. 1 and 2, along with quantification of surface alterations, basophil activation/degranulation can also be studied by measuring phosphorylation of signaling molecules (Ebo et al., 2007; Verweij et al., 2012), exteriorization of granule content (e.g. via avidin probes that bind surface membrane-associated exteriorized anionic proteoglycans released from granules (Ebo et al., 2020c) or more directly via conjugated diamino oxidase in the HistaFlow® (Ebo et al., 2012; Shamji et al., 2015)), intracellular calcium imaging (Heinemann et al., 2003), or quantification of trapped cytokines or mRNA thereof (Ocmant et al.,

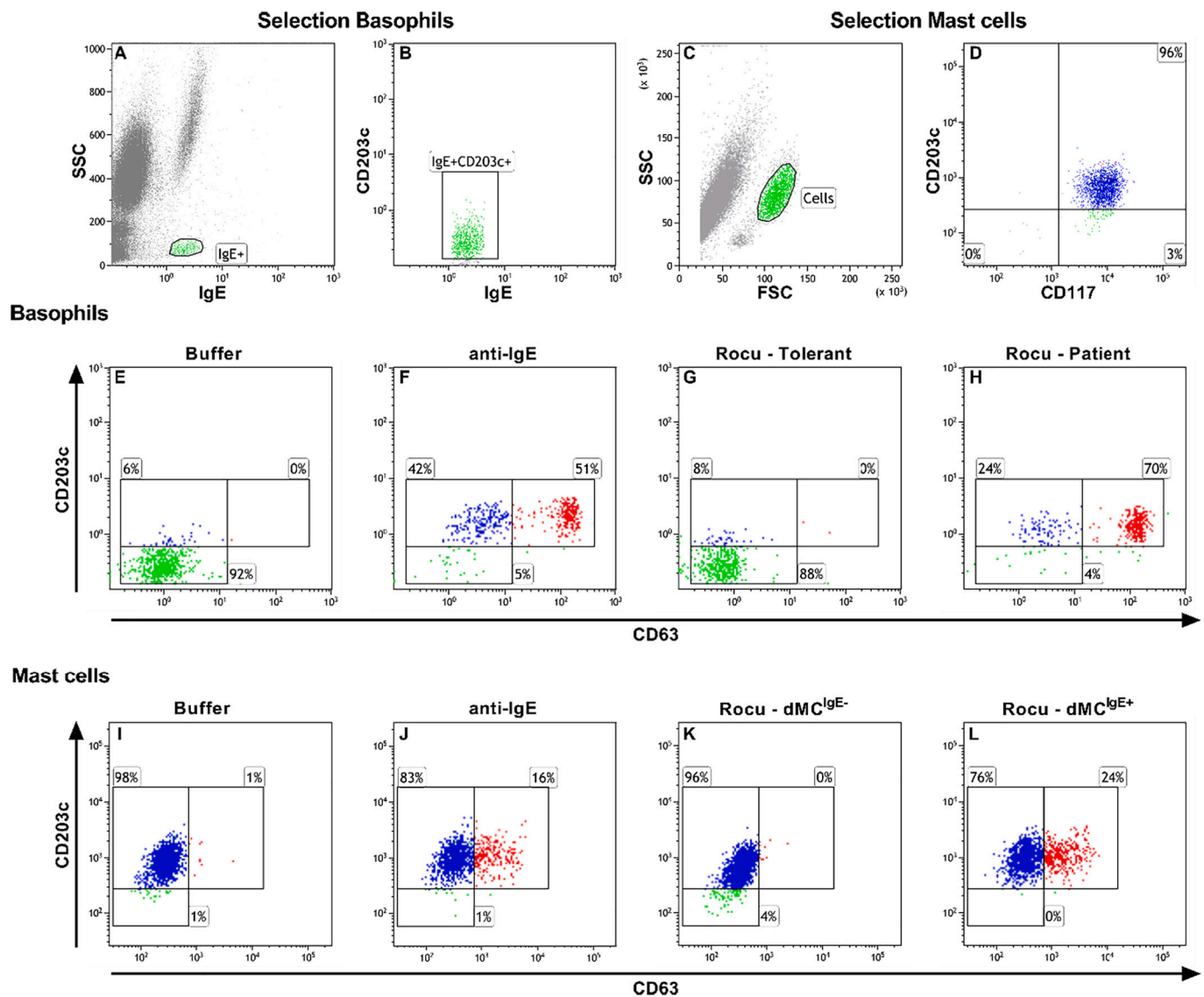


Fig. 4. Activation of basophils and mast cells (MCs) with rocuronium.

(A, B) Basophils are selected based upon scatter characteristics and double positivity for IgE and CD203c ($\text{IgE}^{+ve}\text{CD203c}^{+ve}$). (C) MCs are selected based upon forward scatter (FSC) and side scatter (SSC) characteristics. (D) MCs are defined as $\text{CD117}^{+ve}\text{CD203c}^{+ve}$. (E) Resting basophils (incubated with stimulation buffer) do not degranulate (do not express the lysosomal protein CD63). (F) Expression of CD63 is upregulated after stimulation with the positive control anti-IgE, which cross-links surface-bound IgE antibodies. (G) Basophils of a rocuronium-tolerant individual do not upregulate CD63 in response to rocuronium. Indicating that the cells cannot be stimulated non-specifically. (H) In contrast, basophils of a rocuronium-allergic patient upregulate CD63 after incubation with rocuronium. (I) MCs barely express CD63 after incubation with buffer. (J) MCs that are passively sensitized with serum containing IgE are designated as $\text{dMC}^{\text{IgE}+}$. When these $\text{dMC}^{\text{IgE}+}$ are incubated with anti-IgE, they upregulate CD63. (K) MCs not passively sensitized with patients' serum ($\text{dMC}^{\text{IgE}-}$) do not upregulate CD63 after stimulation with rocuronium. (L) MCs, which are passively sensitized with serum of a rocuronium-allergic patient ($\text{dMC}^{\text{IgE}+}$), upregulate CD63 after stimulation with rocuronium. The end concentration of rocuronium is $164\ \mu\text{M}$. For a more detailed description of our MC culture and the mast cell activation test, the reader is referred elsewhere (Elst et al., 2020b; Elst et al., 2020c; Elst et al., 2020d).

2005; Devouassoux et al., 1999).

3.2. Mast cell activation tests (MATs)

Although BATs have proven to be a significant and safe asset in the diagnosis of IDHRs, the technique also has some weaknesses. These shortcomings, however, can be circumvented by using passive BATs (using stripped basophils that are passively sensitized with specific patient serum) and MATs. However, passively sensitized donor basophils requires stripping of the cells with removal of donor IgE, a procedure that can affect their responsiveness and carries the risk of nonspecific stimulation or damage to basophils and is difficult to standardize (Ebo et al., 2020b; Moneret-Vautrin et al., 1999). Using cultured donor cells

or cell lines (e.g. humanized RBL with transfection of $\text{Fc}\epsilon\text{RI}$ and LAD2 cells), MATs do not require fresh viable cells as classical BATs do. Furthermore, neither passive BATs nor MATs are hampered by a basophilic non-responder status observed in 10–15% of our patients. Finally, because MCs, unlike resting basophils, express the MRGPRX2 receptor, MATs can also facilitate further study of underlying sIgE/ $\text{Fc}\epsilon\text{RI}$ -independent mechanisms such as off-target occupancy of the MRGPRX2. Fig. 1 shows potential read-outs for MAT. Fig. 3 displays confocal microscopy images of resting MCs and MCs stimulated with substance P, the natural ligand of MRGPRX2. As shown in Fig. 4, it suffices to compare degranulation from passively sensitized donor MCs ($\text{dMCs}^{\text{IgE}+}$) with outcomes in cells that were not incubated with patients' serum ($\text{dMCs}^{\text{IgE}-}$), along with selective silencing experiments to confirm

Table 1BAT in immediate β -lactam antibiotic hypersensitivity (data for CD63-BAT, except if denoted in *italics*). Actualized from Decuyper et al. (2017); Mayorga et al. (2017).

Drug	Reference test	Activation marker	Sensitivity	Specificity	Number of patients and controls	PPV	NPV	Reference
β -LABs	H	CD63	50	93	88	93.5	49.1	Sanz et al. (2002)
β -LABs	H + DPT	CD63	39	93	53	/	/	Gamboa et al. (2004)
β -LABs	H $\pm$ ST $\pm$ sIgE $\pm$ DPT	CD63	49	91	110	92.4	50.8	Torres et al. (2004)
AMX	H $\pm$ ST	CD203c	52	100	41	85.2	52.5	Abuaf et al. (2008)
		CD63	22	79				
β -LABs	H	CD63	50	89–97	262	90.5	43.8	De Week et al. (2009)
β -LABs	H $\pm$ ST $\pm$ sIgE	CD63-CCR3	55	100	39	81.5	52.6	Eberlein et al. (2010)
		CD63-IgE	53					
AMX	H	CD63	29	/	14 patients, no controls	/	/	Garcia-Ortega and Marin (2010)
AMX	H $\pm$ ST $\pm$ DPT	CD63	50	/	61 patients, number of controls not mentioned	/	/	Torres et al. (2010)
AMX	H $\pm$ ST	CD63	50	/	30 patients	/	/	Torres et al. (2011)
AMX/CLAV	H $\pm$ ST $\pm$ DPT	CD63	47	93	57	/	/	Salas et al. (2018)
			62	89	58			
CEF	H + ST	CD63	33	94	16 patients, 17 controls	/	/	Uyttbroeck et al. (2016)
		CD203c	67	94				

 β -LABs: β -lactam antibiotics (pooling of patients sensitized to different β -LABs), AMX: amoxicillin, CEF: cefazolin.

H: history, ST: skin test, sIgE: specific IgE, DPT: drug provocation test/Not determined, PPV: positive predictive value, NPV: negative predictive value.

Table 2BAT in immediate neuromuscular blocking agent (NMBA) hypersensitivity (data for CD63-BAT, except if denoted in *italics*). Actualized from McNeil et al. (2015); Decuyper et al. (2017); Ebo et al. (2020a).

Stimulus	Reference Test	Activation marker	Sensitivity	Specificity	N	PPV	NPV	Reference
NMBAs	H + ST	CD63	64	81	26	86.9	78.1	Abuaf et al. (1999)
		CD45	43	96				
NMBAs	H $\pm$ ST	CD63	54	100	56	100	48.6	Monneret et al. (2002)
NMBAs	H + ST	CD63	79	100	31	100	76.9	Sudheer et al. (2005)
		CD203c	36	100				
NMBAs	H $\pm$ ST	CD63	36–86 ^a	93	92	/	/	Kvedariene et al. (2006)
Rocuronium	H + ST	CD63	92 ^b	100	22	100	87.3	Ebo et al. (2006)
NMBAs	H $\pm$ ST $\pm$ sIgE	CD63	60	100	92	84.9	58.3	Sainte-Laudry and Orsel (2008)
Rocuronium	H + ST	CD63	80	96	104	97	75	Leysen et al. (2011)
NMBAs	H + ST	CD63	68	100	56	100	82.9	Hagau et al. (2013)
Atracurium	H + ST	CD63	71 ^c	100	75	/	/	Uyttbroeck et al. (2014)
Rocuronium	H + ST	DAO	100	100	13	100	75	Cop et al. (2016)
NMBAs ^{d,e}	H $\pm$ ST $\pm$ sIgE	CD63	48	87	120	/	/	Li et al. (2019)
		CD203c	58	89				

H: history, ST: skin test, N: number of patients and control individuals. *Italics*: CD203c-BAT.

DAO: conjugated diamine oxidase was used to measure intracellular histamine content (proof-of-concept in IDHR) (Cop et al., 2016).

In the study by Leysen et al. (2011), the PPV for ST and sIgE rocuronium was 98% and 83%; the NPV for ST and sIgE rocuronium was 96% and 72%.

ST: skin test, sIgE: specific IgE, PPV: positive predictive value, NPV: negative predictive value.

^a Increasing sensitivity when only the reactions that occurred during the last 3 years were taken into account.^b Taking into account the non-responders sensitivity is 76%.^c Taking into account the non-responders sensitivity is 63%.^d Sensitivity and specificity for %CD63 and %CD203c. Overall sensitivity and specificity are 77% and 76%, respectively.^e BAT also positive in some patients with negative skin tests (note that CD300a was used in some cases). BAT was also found to supplement skin tests in Van Gasse et al. (2019).**Table 3**BAT fluoroquinolone (FQ) hypersensitivity (data for CD63-BAT, except if denoted in *italics*). Actualized from (Decuyper et al., 2017; Mayorga et al., 2017).

Stimulus	Reference Test	Activation marker	Sensitivity	Specificity	N	PPV	NPV	Reference
FQs	H + DPT	CD63	0	–	4	/	/	Seitz et al. (2009)
FQs	H + ST + DPT	CD63	0	100	18			Lobera et al. (2010)
FQs	H	CD203c	100	100	5	/	/	Ben Said et al. (2010)
FQs	H + DPT	CD63	71	–	75	90.1	66.7	Aranda et al. (2011)
FQs	H + DPT	CD203c	NA	100	34	100	78.9	Rouzaire et al. (2012)
Moxifloxacin Ciprofloxacin	H + DPT	CD63	9.1	77.8	11	81.8	66.7	Fernandez et al. (2016)
		CD203c	36.4	94.4	11			
		CD63	83.8	88.9	6			
		CD203c	0	94.4	6			
Moxifloxacin	H	CD63	13.3	100	24			Van Gasse et al. (2017a)
		CD203c	46.7	100	24			

H: history, ST: skin test, DPT: drug provocation test, N: number of patients and control individuals.

Italics: CD203c-BAT.

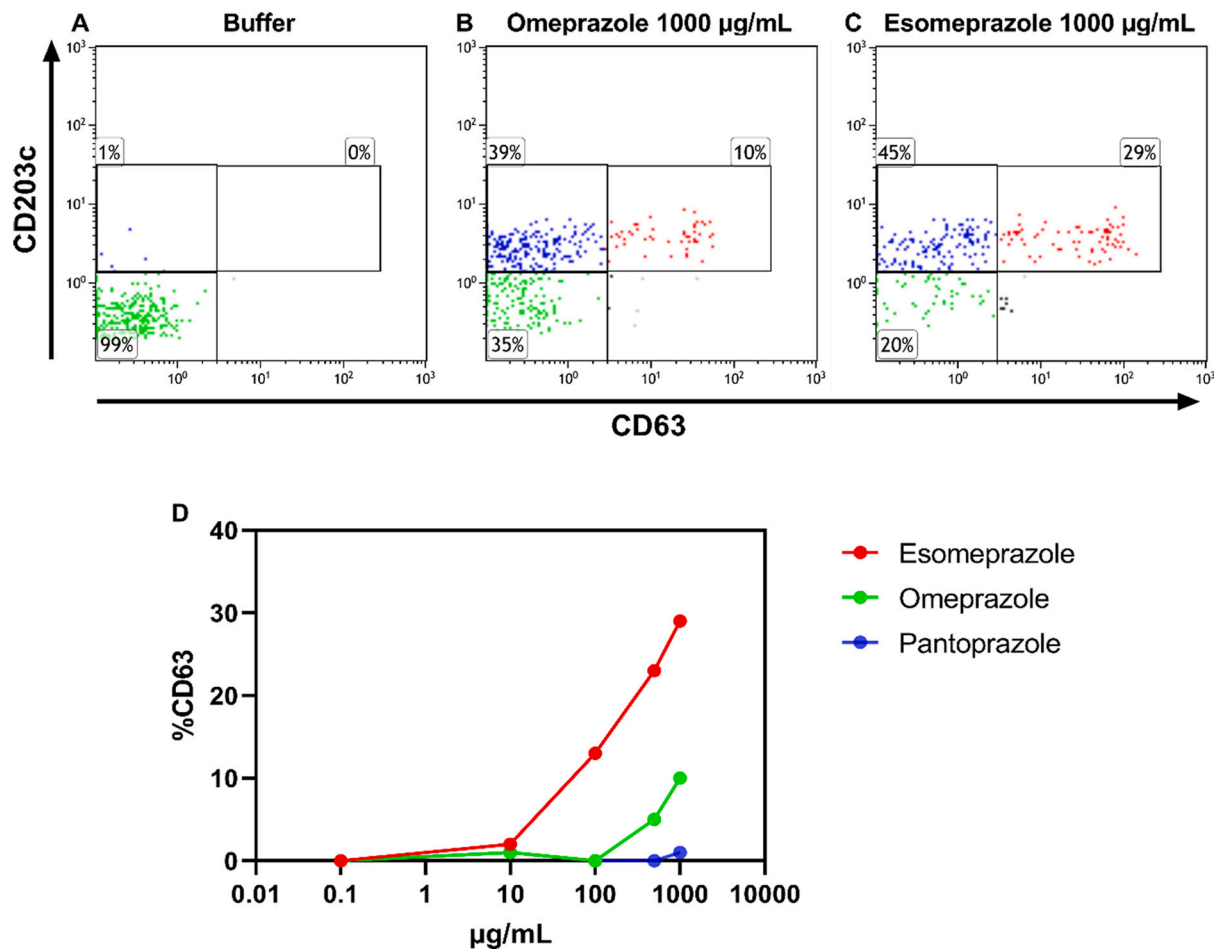


Fig. 5. Representative plots of basophil activation test for omeprazole, esomeprazole and pantoprazole in a patient who experienced twice anaphylaxis with generalized urticaria, bronchospasm, nausea and diarrhea within 30 min after intake of omeprazole with etoricoxib. Diagnosis of omeprazole hypersensitivity was suggested by a positive intradermal test for this proton pump inhibitor (wheal/flare 9/12 mm, Losec® 40 mg/mL, 1:100). Skin prick tests at neat solution and intradermal tests 1:10 for esomeprazole (Nexiam®, 40 mg/mL) and pantoprazole (Pantomed®, 40 mg/mL) and oral graded provocation test with etoricoxib (Arcoxia®) up to a cumulative dose of 131 mg were negative. The patient refused provocation tests with pantoprazole. (A) Stimulation with buffer does not induce upregulation of the anaphylactic degranulation marker CD63. (B,C) Stimulation with omeprazole and esomeprazole, respectively. Note the unimodal upregulation of CD203c and bimodal upregulation of CD63. (D) Dose findings for omeprazole, esomeprazole and pantoprazole.

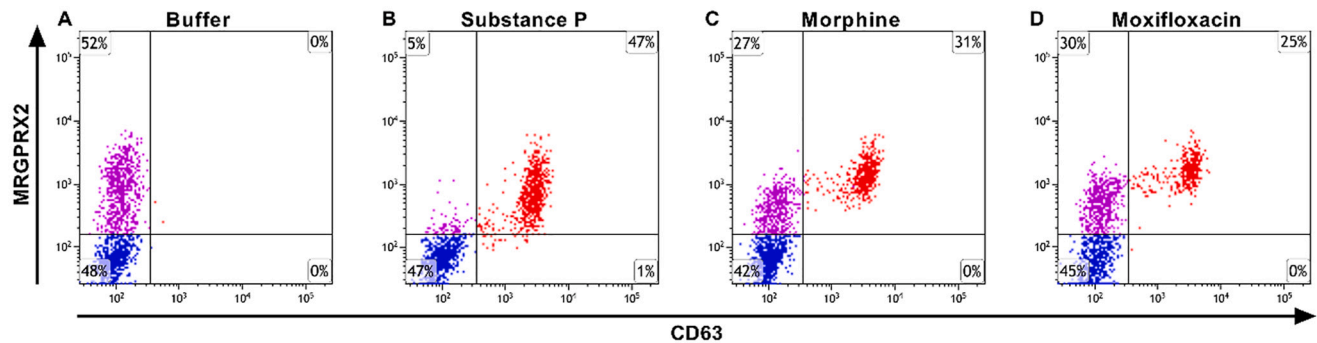
implication of the MRGPRX2 (Elst et al., 2020c). Although similar results can be obtained with RBL stably transfected with MRGPRX2 and specific human MC lines (e.g. LAD2) (Falcone et al., 2018; Santos et al., 2018; Bahri et al., 2018), we, as others (Bahri et al., 2018; Luo et al., 2020), have mainly been using cultured MCs that can be obtained from healthy donors and, if needed, from patients. In our protocol, such donor MCs are obtained after 4 weeks culture starting from CD34⁺ve progenitors out of about 50 mL of donor blood. These MCs, identified as CD117⁺veCD203c⁺ve cells, exhibit a connective tissue MC_{TC} phenotype and thus express tryptase, chymase, histamine and functional surface FcεRI and MRGPRX2 (Elst et al., 2020a; Cop et al., 2017; Cop et al., 2018).

4. Basophil activation tests in immediate drug hypersensitivity: diagnosis and beyond

The applications, limitations and controversies about BATs in IDHRs have been extensively reviewed elsewhere (Ebo et al., 2020b; Decuyper et al., 2017; Takazawa et al., 2019; Mayorga et al., 2019; Mayorga et al., 2017). From these reviews, the BAT has emerged as a well-performing diagnostic test for IDHRs, at least for some classes (summarized in Tables 1–3). For example, for NMBAs, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) generally

vary between 50 and 100%, 85–90%, 85–100% and 55–80%, respectively, and for β-lactam antibiotics, between 30 and 55%, 80–100%, 50–90% and around 55%, respectively. Moreover, in IDHRs to NMBAs and β-lactam antibiotics, BATs detected sensitizations that were missed by skin testing (Van Gasse et al., 2019; Sabato and Ebo, 2018). Similar findings were reported in a single study about proton pump inhibitors (Laguna et al., 2018). For iodinated contrast media, NPV is around 83% and PPV 96% (Mayorga et al., 2017). In contrast, for fluoroquinolones, the results seem highly variable and difficult to interpret. The reasons for these divergent results are diverse and largely relate to the applied protocol and setting of sometimes arbitrarily chosen decision thresholds. The low sensitivity of the traditional CD63-based BATs as reported by various authors (Van Gasse et al., 2017a; Seitz et al., 2009; Lobera et al., 2010) most likely results from the fact that resting whole blood basophils, unlike isolated basophils (Wedi et al., 2020a; Wedi et al., 2020b), barely express the MRGPRX2 receptor (Wedi et al., 2020a; Sabato et al., 2017; Wedi et al., 2020b; Sabato et al., 2020). Therefore, in contrast to our earlier expressed hypothesis (Van Gasse et al., 2017a), we think that resting whole blood basophils are not responsive to fluoroquinolones that are currently believed to trigger IDHRs via this receptor (Porebski et al., 2018; Liu et al., 2019; Van Gasse et al., 2017c). In this respect, it is evident that the BAT can only add to diagnosis in IDHRs that involve degranulation of basophils. It cannot document IDHRs resulting from

Mast cells



Mast cells - silenced with DsiRNA MRGPRX2

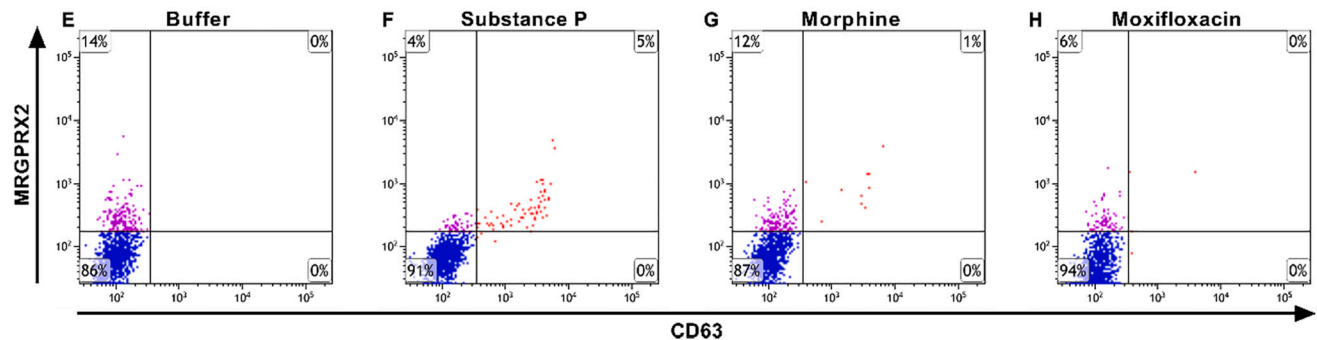


Fig. 6. MRGPRX2 and CD63 expression by mast cells (MCs) (A) and MCs with selective silencing of MRGPRX2 by DsiRNA (E). Resting MCs obtained from CD34⁺ progenitor cells in peripheral blood as described in (Elst et al., 2020a; Elst et al., 2020c; Cop et al., 2017; Elst et al., 2020d) display a MC_{TC}-like phenotype with a MRGPRX2⁺ and MRGPRX2⁺ subpopulation (A). As shown in plots B–D, unlike MRGPRX2⁺ cells, MRGPRX2⁺ cells respond to the neurokinin substance P (the natural ligand of MRGPRX2), the opiate morphine and the fourth generation fluoroquinolone moxifloxacin. As shown in panels F–H, these responses are abolished by knocking out MRGPRX2 surface expression using DsiRNA (Elst et al., 2020c).

alternative mechanisms such as enzymatic inhibition by non-selective cyclo-oxygenase (COX)1,2 inhibitors, as is the case with hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs) and bradykinin-induced angioedema from angiotensin-converting enzyme (ACE) inhibitors (Mayorga et al., 2019). Moreover, neither BAT nor IgE-binding studies, could show any evidence for an IgE-mediated reaction to parent drug or metabolites in patients who experienced selective diclofenac hypersensitivity (Harrer et al., 2010).

Alternatively, the BAT has been shown to be a reliable adjunct in difficult cases, mainly because of uncertainties associated with drug-specific IgE assays and/or skin testing. For example, in contrast to sIgE and skin testing (Van Gasse et al., 2015; van der Poorten et al., 2020; Nasser and Ewan, 2001), BATs can help to resolve between sIgE/FcεRI and non-specific – likely MRGPRX2-dependent – opiate hypersensitivity, as resting basophils, unlike MCs barely express MRGPRX2 (Ebo et al., 2020a; Laysen et al., 2013). Another advantage of the BAT lies in its capacity to simultaneously and safely test different compounds, including products that are not intended for human use. This asset is particularly useful when exploring the absence or presence of cross-reactivity (Laysen et al., 2013; Ebo et al., 2006; Ebo et al., 2019a; Beyaz et al., 2020; Philipse et al., 2013), incrimination of (hidden) excipients (Ebo et al., 2001; Dumond et al., 2009; Walker et al., 2011; Rodríguez Trabado et al., 2017), identification of specific antigenic determinants or changes thereof (Barbero et al., 2019; Ebo et al., 2019b). For example, we used the BAT to demonstrate that the sugammadex-rocuronium inclusion complex (S-R-Cx) can be allergenic and is capable of triggering sIgE/FcεRI-dependent anaphylaxis due to a shape alteration involving the thio(carboxyethyl)sodium side chains attached at the primary rim of the host sugammadex molecule. Since these changes appeared not specific to the guest rocuronium, the use of sugammadex as a selective relaxant-reversal agent for other NMBA had

to be precluded in that particular case. Other applications of BATs, such as the follow-up of allergen-specific immunotherapy and desensitization strategies in IDHRs, have been summarized in (Ebo et al., 2020d). Fig. 5 shows a representative plot for omeprazole, esomeprazole and pantoprazole in a patient who experienced anaphylaxis from the proton pump inhibitor omeprazole. Diagnosis was documented by a positive intradermal test with a wheal/surrounding flare of 9/12 mm for omeprazole 1:100 (Losec®, 40 mg/mL). Interestingly, in contrast to skin testing, the BAT was suggestive of cross-reactivity between omeprazole and esomeprazole, a frequent phenomenon (Tourillon et al., 2019). The finding, which was obtained when the BAT was used as a complementary test to skin testing in the diagnosis of proton pump inhibitor hypersensitivity, is in line with the findings by Laguna et al. (Laguna et al., 2018), who reported that the BAT was positive in 57.1% of the patients with negative skin tests in 42 patients who had undergone skin testing or had been diagnosed with provocation proven omeprazole hypersensitivity.

5. Mast cell activation tests in immediate drug hypersensitivity: filling an unmet need?

Although the BAT has proven to be a performant diagnostic test in IDHRs that adds to our understandings of the underlying mechanistic processes of IDHRs, there is still room for improvement. As discussed above, the BAT does not allow deferred testing, since it requires processing within 4 h of collection and is lost as a diagnostic in 10 to 15% of individuals because of a non-responder status of the cells (i.e. basophils do not respond to the positive control or to the allergen). One strategy that has been adopted to circumvent these weaknesses, consists in using cell lines such as humanized rat basophilic leukemic cells (RBL-2H3 cells) or human leukemic MCs (LAD2 cells) (Falcone et al., 2018; Santos

et al., 2018; Bahri et al., 2018; Passante, 2020). However, these studies have shown that LAD2 cells have lower analytical sensitivity than traditional BATs and MATs which use dMCs^{IgE+} passively sensitized with patients' sera (Santos et al., 2018; Bahri et al., 2018), suggesting that dMCs^{IgE+} might be more suitable than LAD2 cells in IgE/FcεRI-mediated degranulation studies. In addition, it was shown that flow-assisted MATs using dMCs^{IgE+} confer superior diagnostic accuracy compared with existing diagnostics in discriminating between patients with positive and negative challenge results, and that the MAT response is not exclusively dependent on sIgE titers but likely also reflects antibody affinity and/or interindividual variations in antibody-antigen specificities. However, the development of a flow-assisted MAT using dMCs^{IgE+} for drug hypersensitivity is still ongoing and currently limited to our proof of concept in chlorhexidine (CHX) hypersensitivity (Elst et al., 2020d). Chlorhexidine allergy, a sIgE/FcεRI-mediated condition *par excellence*, has grown into one of the most important and too frequently overlooked causes of perioperative hypersensitivity and anaphylaxis (Rose et al., 2019). For example, in our series, CHX is the fourth most important cause of perioperative anaphylaxis after NMBA, latex and cefazolin. Generally, diagnosis of CHX allergy rests upon quantification of CHX-reactive sIgE antibodies and CHX skin testing. However, in difficult cases, displaying equivocal or negative results, BATs and MATs using dMCs^{IgE+} might benefit diagnosis. Moreover, we have shown that the MAT has a high analytical sensitivity, since successful passive sensitization was obtained for titers of CHX-reactive sIgE as low as 0.66 kUA/L in the traditional ImmunoCAP assay, and that it also displays superior performance in distinguishing between patients with true CHX allergy and those who are sensitized but most likely tolerant (as indicated by incongruent positive CHX-sIgE results and negative skin testing and BAT for CHX). Whether these findings will result in novel diagnostics remains to be seen. However, as illustrated in Fig. 4 preliminary results indicate that this technique might also be applicable for the aminosteroid-derived NMBA rocuronium. Moreover, this figure also seems to indicate that a comparative analysis between basophils, dMCs^{IgE+} and dMCs that were not passively sensitized with patients' IgE (designated as dMC^{IgE-}), combined with targeted gene silencing, showed in Fig. 6, might represent a powerful approach to further unveil the mechanisms beyond IDHRs resulting from cross-linking of sIgE/FcεRI complexes or occupation of MRGPRX2 and to explore differences in effector cell function between basophils and MCs.

6. Conclusion

IDHRs constitute a significant health issue with serious consequences of diagnostic error. Unfortunately, correct diagnosis of IDHRs may pose a difficult challenge, mainly because of uncertainties and limitations associated with existing diagnostics and unknowns about pathomechanisms. Over the last 25 years, flow-assisted BATs have emerged as a robust tool to diagnose IDHRs - at least for some drug classes -, to study cross-reactivity between drugs and related compounds, to advance our knowledge and understandings of basophil function during IDHRs, and to monitor the effects of desensitization strategies. However, BATs are technically challenging and currently limited to a few specialized laboratories, require fresh samples and are lost as a diagnostic in patients showing a non-responder status of their cells. In contrast, MATs use serum samples that can be frozen, stored, and shipped to a reference centre experienced in MC lines and/or cultures, and allow (deferred) batch testing with necessary quality controls. Although currently restricted to a proof of concept and some preliminary results, the MAT appears to be highly sensitive and to have good discrimination performance between true drug allergy and cases with indeterminate diagnostics (e.g. sensitization as reflected by an isolated positive sIgE result). Finally, the MAT, especially when performed in combination with basophil experiments, appears to be an attractive complementary method to further accelerate our insight into the effector mechanisms of IDHRs.

Declaration of Competing Interest

The authors declare no conflict of interest.

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