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Are we ready to downregulate mast cells?

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Downregulation of mast cells (MCs) function and/or survival is warranted in allergic inflammation (AI), mastocytosis/MC leukemias and in other inflammatory diseases in which MCs have a central role. Human MCs (hMCs) have been recently shown to express the death receptor (DR) TRAIL and the inhibitory receptors (IRs) CD300a and Siglec-8.TRAIL is the only known DR functional on hMCs, and interestingly its function is upregulated by IgE-dependent MC activation. The newly described IRs, CD300a and Siglec-8, potently downregulate MC activation and survival *in vitro* and inhibit different IgE-mediated responses *in vivo*. Therefore a selective targeting of TRAIL and of IRs on MC could be a novel immunopharmacological way to downregulate MC-associated diseases.

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Introduction

The key effector cells of allergic inflammation (AI) are the MCs and hence their downregulation is considered to be one of the main ways to inhibit allergic diseases. It is conceivable that during an AI response MCs are influenced by a number of activating and inhibitory signals. Among the first ones those that are transduced by FcεRI and c-Kit, and among the second, those transduced by IRs are the most typical. MCs are long living cells [1] that survive repetitive activation, an event that stimulates their biochemical regeneration [2,3]. Therefore, one of the modalities to end MC function could be the induction of their apoptotic death by DRs. However, until recently no DRs have been shown for hMCs. It is now being demonstrated that these cells do express functional TRAIL. A more 'classical' way than DRs to downregulate MCs is via IRs. The first discovered and most studied IRs on hMCs is FcγRIIB. Additional IRs have been found on hMCs and CD300a and Siglec-8 are among the most promising ones. Therefore, although MC survival/function might be regulated by additional suppressing mechanisms, such as specific cytokines or lipid mediators [4], it seems that targeting MC may be more feasible via DRs or IRs. The scope of this paper is to review new information on hMC DRs and IRs, and to discuss them as possible therapeutic targets in allergy and MC-related diseases.

DRs on MC

Apoptosis or programmed cell death is an intrinsic mechanism of cell death often used to end an immune response [5,6]. Two main signaling pathways initiate the apoptotic program: the mitochondrial/intrinsic one and the DRs/extrinsic one. Both pathways rely on a family of intracellular cysteine proteases called caspases [7]. DRs on hMCs have not been studied and until recently most of the information was based on murine MCs (mMCs). Nevertheless, hMCs have been demonstrated to undergo the intrinsic apoptotic pathway when deprived of stem cell factor (SCF) [8–10]. The expression of two DRs, FAS/CD95R and TRAIL-R (TNF-related apoptosis inducing ligand TRAIL/Apo-2L-receptor), belonging to the tumor-necrosis factor (TNF) receptor superfamily, was identified in mMCs and hMCs.

TRAIL-R and FAS/CD95R

Five different TRAIL-Rs have been identified, but only two of them, that is DR4 and DR5 are capable of apoptotic signal transmission [11]. Both receptors are type I transmembrane proteins with a C-terminal intracellular tail containing a cytoplasmic domain of about 80 amino acids called 'the death domain'. The binding of TRAIL leads to trimerization and hence activation of the DR-mediated death pathway [12].

TRAIL receptors are found on cells involved in AI such as neutrophils, where they disrupt anti-apoptotic pathways initiated by survival factors [13], whereas on eosinophils they promote cell survival [14,15]. Recently TRAIL-R was identified in the leukemia MC line HMC-1 [16], in primary human lung-derived MCs (hLMCs) and in cord-blood-derived MCs (CBMCs). The cross-linking of the receptor by TRAIL trimer caused activation of 'executioner' caspase-3 and a significant increase in CBMC apoptosis [17••]. Moreover, it caused a significant change

Table 1

DRs expressed on murine and human mast cells

Receptor	Human MC	Murine MC	Expression regulation[a]	Functionality regulation[a]	Ligand
TRAIL-R1/DR4	CBMC HMC LAD	Unknown	Unknown	Unknown	TRAIL
TRAIL-R2/DR5	CBMC HMC LAD	Unknown	FcεRI cross-linking ↑	FcεRI cross-linking ↑	
FAS/CD95R	hLMC	BMMC peritoneal MC lines: C57, MCP-5, MC9	IL-4+IL-10/IL-4 ↑	IL-4+IL-10/IL-4 ↑	FAS
	HMC-1			FcεRI or FcγR cross-linking ↓	

[a] MC activation by FcεRI or FcγR cross-linking or treatment with Th2 cytokines upregulates (↑) or downregulates (↓) expression and function of DRs.

in mitochondrial membrane potential and the truncation and consequent activation of the proapoptotic protein BID. An increase in the expression level of non-truncated BID was observed as well [18•]. These findings suggest that in addition to the extrinsic pathway, in hMCs, TRAIL involves also activation of the intrinsic one.

Importantly, the IgE-dependent activation of hMCs enhanced TRAIL-induced 'initiator' caspase-8 and 'executioner' caspase-3 cleavage and increased their susceptibility to TRAIL-induced apoptosis [18•]. However, the molecular mechanism responsible for this effect is not clearly understood as yet.

Indeed, in CBMCs as well as in tonsil-derived hMCs and hLMC, IgE-dependent activation increases TRAIL–R2/DR5 expression levels (Table 1) [17••,19]. However, as demonstrated also in mMCs [20], IgE-dependent activation of CBMCs simultaneously causes an increase in prosurvival molecules, that is FLICE/caspase-8 inhibitory protein (FLIP) and myeloid cell leukemia-1 (MCL-1) [16] as well as in proapoptotic BIM expression [17••].

FAS/CD95R was found to be expressed on primary and transformed mMCs. C57 and MC/9 mMCs are susceptible to FAS apoptotic killing, an event that is upregulated by Th2 cytokines [21–24], but can be abrogated by MC activation [23]. Although identified on HMC-1 and hLMC, so far FAS was found not to be functional in these cells (Table 1) [17••]. Therefore, it is important to further study the possible regulation of FAS expression and functions on hMCs.

Inhibitory receptors (IRs)

One of the mechanisms used by the immune system to avoid excessive responses is its downregulation by IR containing immunoreceptor tyrosine based inhibitory motifs (ITIMs). IRs have been discovered and characterized on various cell types, especially on NK cells but also on other hematopoeitic cells.

IRs belong either to the Ig receptor super family characterized by a single V-type Ig-like domain in the extracellular portion, or to the c-type (calcium-dependent) lectin super family [25,26]. Upon activation, these receptors undergo tyrosine phosphorylation, often by a kinase of the Src family, which provides a docking site for the recruitment of cytoplasmic phosphatases having an SH2 domain such as SHP-1, SHP-2 and SHIP.

So far, a number of IRs have been identified on MC both of human and rodent origin. IRs associated exclusively with rodent MC such as gp49B1, PIR-B, MAIR-I and MAFA have been recently reviewed [27–30]. We will focus on hMC IRs that include FcγRIIB, CD300a, Siglec-8, LIRs, LAIRs, SIRP-α and CD200 (Table 2). Since

Table 2

Inhibitory receptors expressed on hMCs

Receptor	Cell distribution other than MC	Number of ITIMs or ITIM-like domains
FcγRIIB	B cells, myeloid	1
CD300a	NK, T, pDC, granulocytes	4
Siglec-8	Eosinophils, basophils	2
LAIR-1	Eosinophils, basophils, neutrophils, NK, B cells, mononuclear phagocytes	2
SIRP-α	Dentric cells, mononuclear phagocytes, basophils	4
CD200R	Myeloid, lymphoid, neuronal	None[a]

[a] Does not contain ITIM; contains 3 tyrosine residues.

FcγRIIB has been widely and recently reviewed [31,32,33••] we will examine mainly the other IRs.

CD300a

CD300a (IRp60 or CRMF-35), belonging to the Ig superfamily, is expressed on NK cells, MCs, T cell subsets, granulocytes, monocytes and dendritic cells (Table 2) [34–37]. Its co-aggregation on NK cells results in downregulation of their cytolytic activity. CD300a contains four ITIMs in its cytoplasmic tail, three of them being classical and the fourth is non-canonical [34].

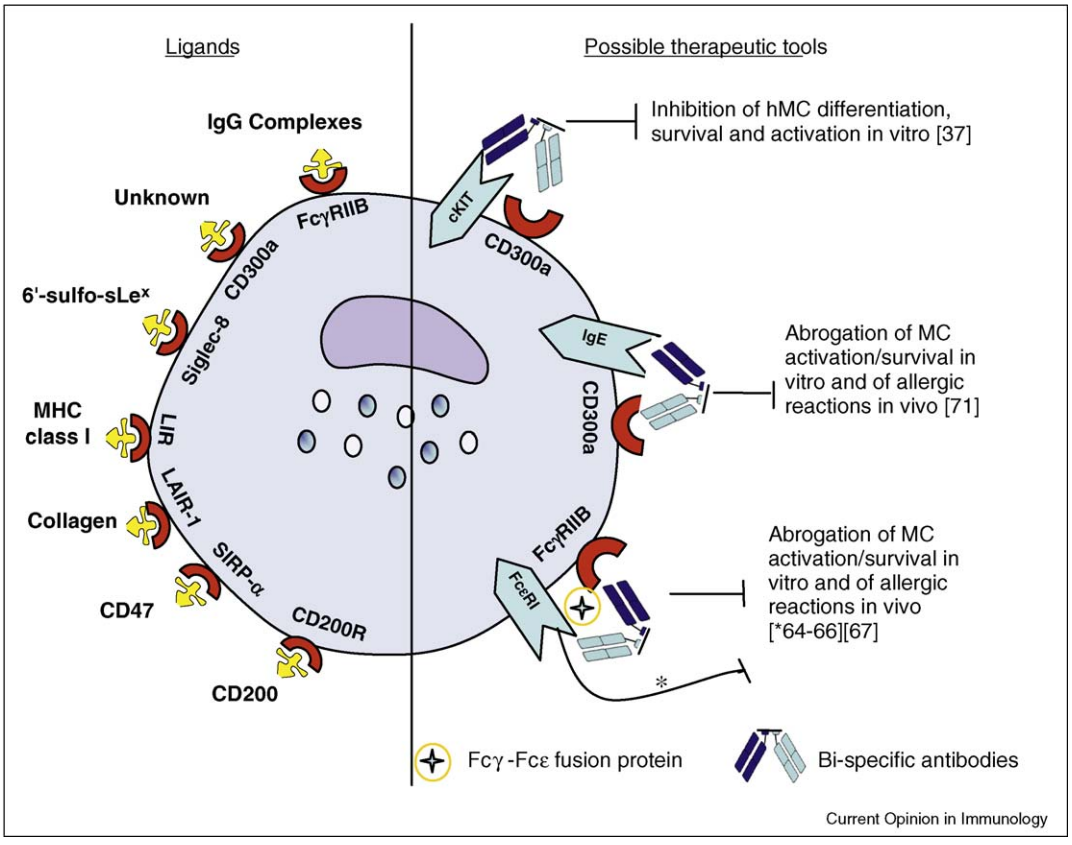
CD300a co-aggregation with IgE-bound FcεRI on CBMC, leads to inhibition of Ig-E induced (but not of compound 48/80 induced) β-hexosaminidase, tryptase and IL-4 release. Concomitantly, an increase in CD300a phosphorylation, recruitment of SHP-1 and SHIP-1, decrease in [Ca^2+] influx and increase in syk dephosphorylation were detected. Moreover CD300a cross-linking inhibited SCF-mediated CBMC survival [35]. It is important to point out that FcγRIIB, when co-ligated to FcεRI, inhibits MC degranulation and cytokine production by recruiting SHIP-1 but not SHP-1/2 [33••,38–40].

Human eosinophils and neutrophils also express CD300a. On eosinophils, CD300a engagement was able to suppress the activation effects of eotaxin, IL-5 and GM–CSF by recruiting SHP-1 phosphatase [36].

On neutrophils, CD300a is upregulated by LPS or GM-CSF and leads to the inhibition of FcγRIIa-mediated activation and ROS production, but not of TLR-4-mediated ROS production [37].

Linking CD300a with c-Kit by a bi-specific antibody recognizing both receptors, abrogated c-Kit-mediated CBMC differentiation, survival, activation and also IgE-dependent activation (Figure 1).When added to the HMC-1, where c-Kit is constitutively activated, it inhibited mediator release with no effect on their survival. In CBMC the bi-specific antibody induced CD300a rapid phosphorylation and recruitment of SHIP-1, but not of SHP-1. Importantly, CD300a activation did not dephosphorylate c-Kit, but it did so on Syk and LAT kinases,

Figure 1



Inhibitory receptors and their targeting. Some of the prominent hMC IRs with their ligands and the immunopharmacological tools for their downregulation already assessed *in vitro* and *in vivo* models of allergy.

eventually leading to their deactivation [41••]. Similarly, FcγRIIB negatively regulated c-Kit-dependent BMMC proliferation when co-aggregated with c-Kit [42].

CD300a has an activating counterpart CD300c that shares great homology with CD300a. Both have been found on pDCs, where they are pivotal in the regulation of TNF-α and IFN-α secretion mediated by TLR-7 and TLR-9 [43].

CD300a and other members of this family have mouse orthologs named CLM–CMRF-like molecules. The murine homolog of CD300a, LMIR-1 (or CLM-8), shares almost 80% homology with the human receptor and is capable of recruiting SHP-1, SHP-2 and SHIP [44].

The ligand for CD300a, as well as for the other members of the CD300 family, remains unknown. Evolutionary data reveal that CD300a is one of the human genes that show strong positive selection [45,46] hinting that its potential ligand has gone through a strong positive selection as well [47••].

Relevant to disease, CD300a and CD300c expression is altered on T cells and pDCs in patients with psoriasis. Interestingly, the CD300 gene complex is linked to PSORS2, a psoriasis susceptibility locus [48] also linked to atopic dermatitis and rheumatoid arthritis [49,50]. Still, further investigation is needed to understand the cause of the alterations in the surface expression and function of the CD300 family members and to see if these changes exist in other inflammatory diseases such as allergy.

Siglec-8

In humans, the sialic acid-binding immunoglobulin-like lectin (siglec) protein family consists of more than 10 members. hMCs express Siglec-2, Siglec-3, Siglec-5, Siglec-6, Siglec-8 and Siglec-10 [51]. Siglec-8 was identified on eosinophils and thought to be specific for these cells [52]. Later on, it was found on basophils and MCs [53] and to date, is the most studied siglec in hMCs. The cytoplasmic tail of Siglec-8 contains two ITIM-like domains that upon antibody-induced co-ligation of the receptor, undergo activation, recruit SHP-1 and trigger down stream inhibitory events. This, in eosinophils, leads to their apoptotic cell death [54,55]. Siglec-8 appears on hMCs at the same time as other MC markers such as FcεRI. When cross-linked by mAbs on peripheral blood-derived MCs, Siglec-8 does not lead to apoptosis, but rather to strong inhibition of histamine and prostaglandin D_2 secretion and of [Ca^{2+}] influx [56]. Interestingly, no effect was found on the release of at least one newly produced cytokine, IL-8, in these cells [57•].

In the murine system, Siglec-F should be regarded as the counterpart for Siglec-8, even though Siglec-F is expressed mainly on mouse eosinophils and not on mMCs. However, both Siglec-8 and Siglec-F specifically recognize the sialoside sequence 6′-sulfo-sLe^x [58]. The natural ligands for Siglec-8 and Siglec-F are still unknown. Approaches using Siglec-F and Siglec-8 Ig fusion proteins, found selective binding to airway epithelial cells and to lung mononuclear cells, which produce sLe^x structures. Therefore, mucins have been proposed as their potential ligands. Indeed a recent work has shown the ability of these mucins to engage with siglecs and to induce apoptosis on monocytes [59•].

Other human MC IRs

hMCs, as well as other hematopoeitic cells express the IRs LIRs, LAIRs, SIRP-α and CD200 all belonging to the Ig-superfamily [60,61]. The LIRs and SIRP-α usually contain three to four ITIMs or ITIM-like sequences whereas LAIR-1 contains two (Table 2). LIRs/LAIRs usually associate with SHP-1 while SIRP-α, recruits both SHP-1 and SHP-2 [27]. In all of these cases, this association with phosphatases leads to an inhibition in [Ca^{2+}] influx, and finally to the decrease in the release of mediators and cytokines [61].

The ligands for LIRs are MHC class I molecules. Regarding LAIR-1, it has been thought for a while that it binds the epithelial cell adhesion molecule Ep–CAM similarly to the murine gp49B1 (that has been shown to bind αvβ3 integrin) [27,62]. This thought was refuted and it is now known that collagens are the functional ligands for LAIR-1 [63]. SIRP-α binds to the integrin-associated protein CD47, found on various cell types.

As opposed to these classical ITIM containing IRs, CD200R, a member of the Ig supergene family lacks an ITIM, and instead it contains three tyrosine residues in its cytoplasmic domains (Table 2) [64,65]. Engagement of CD200R by its ligand (CD200) or by antibodies, results in phosphorylation and Dok1/2 and SHIP recruitment. It eventually leads to an inhibition of hMC and mMC degranulation and cytokine production. This response apparently does not require the co-ligation of this receptor to an activating receptor such as FcεRI [66,67].

Therapeutic potential of DRs and IRs

The expression of DRs and IRs on hMCs makes them an attractive target for drugs for allergy and other MC-driven diseases. The discovery of TRAIL-R on hMCs is very novel, and the fact that this receptor is not expressed in mMCs and FAS does not induce apoptosis in hMCs, renders the research in this direction intriguing. On the contrary, studies on IRs aimed for a therapeutical development, are at a more advanced stage.

Following the discovery of the first IR on MC, FcγRIIB bi-specific antibodies recognizing both FcεRI and FcγRIIB were generated and found to be suppressive on human basophils and hMC activation (Figure 1) [68].

The next step was the recombinant hFcγ–hFcε fusion protein (hGE2-linking FCεRI with FcγRIIB). hGE2 showed inhibitory effects *in vitro* and *in vivo* in a murine passive cutaneous anaphylaxis (PCA) model and in skin test of rhesus macaques, naturally allergic to the dust mite *D. farinae* [69,70]. In a more recent research, hGE2 was administrated therapeutically to *A. suum*-sensitized cynomolgus monkeys and protected the animals from skin anaphylaxis for three weeks, thereby marking hGE2 as a candidate for future treatment of allergic diseases [71].

As with FcγRIIB, CD300a is another promising target for allergy treatment. Therefore, one bi-specific antibody linking CD300a to IgE-bound FcεRI (specific for MCs and basophils) (Figure 1) and another linking CD300a and CCR3 (specific for eosinophils), have been generated by chemical synthesis. When tested on hMCs and eosinophils *in vitro* for their efficacy, they were found to be active in downregulating the activation of these cells [72] [73].

Moreover, when evaluated in murine models of allergic peritonitis, PCA and acute and chronic asthma, murine anti-CD300a/anti-IgE and anti-CD300a/anti-CCR3 bi-specific antibodies, proved to be effective in abrogating these allergic reactions [73].

Regarding CD200R, pre-treatment of mice with the anti-mCD200 mAbs 24 h before PCA induction, inhibited the PCA reaction in a dose-dependent fashion [65].

The recent discovery of the rather selective expression of Siglec-8 on eosinophils and hMCs, makes Siglec-8 the next promising potential IR target for allergy.

Conclusions

hMCs express TRAIL-R and the newly discovered CD300a and Siglec-8, together with the previously found FcγRIIB and other IRs. When activated, DRs induce hMC apoptosis and IRs, inhibition of activation and survival.

Fusion molecules or bi-specific antibodies linking IR with activating receptors have been shown to have a potential therapeutic role *in vitro* and in animal models of allergy. It is feasible to predict that TRAIL-R can also be selectively activated on hMCs to induce apoptosis, as was demonstrated for example with bi-specific antibodies for FAS and the neuronal glial antigen-2 on glioblastoma cells [74]. The next research challenges in these directions are to define the regulation, expression and function of DRs and IRs in disease, and their interaction with drugs typically used in allergy. Moreover, as in the specific case of CD300a, there is a need to discover its ligand(s). Finally, although MC-directed therapy might not be the only answer to treat/prevent AI, at present it looks the most promising one and should therefore be seeked thoroughly.

Conflicts of interest

The authors declare no conflict of interest.

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