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REVIEW

CD300 family receptors regulate eosinophil survival, chemotaxis, and effector functions

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Abstract

The CD300 family of receptors is an evolutionary conserved receptor family that belongs to the Ig superfamily and is expressed predominantly by the myeloid lineage. Over the past couple of years, accumulating data have shown that eosinophils express various Ig superfamily receptors that regulate key checkpoints in their biology including their maturation, transition from the bone marrow to the peripheral blood, migration, adhesion, survival, and effector functions in response to numerous activating signals such as IL-4, IL-33, and bacteria. In this review, we will present the emerging roles of CD300 family receptors and specifically CD300a and CD300f in the regulation of these eosinophil activities. The structure and expression pattern of these molecules will be discussed and their involvement in suppressing or co-activating eosinophil functions in health and disease will be illustrated.

KEYWORDS
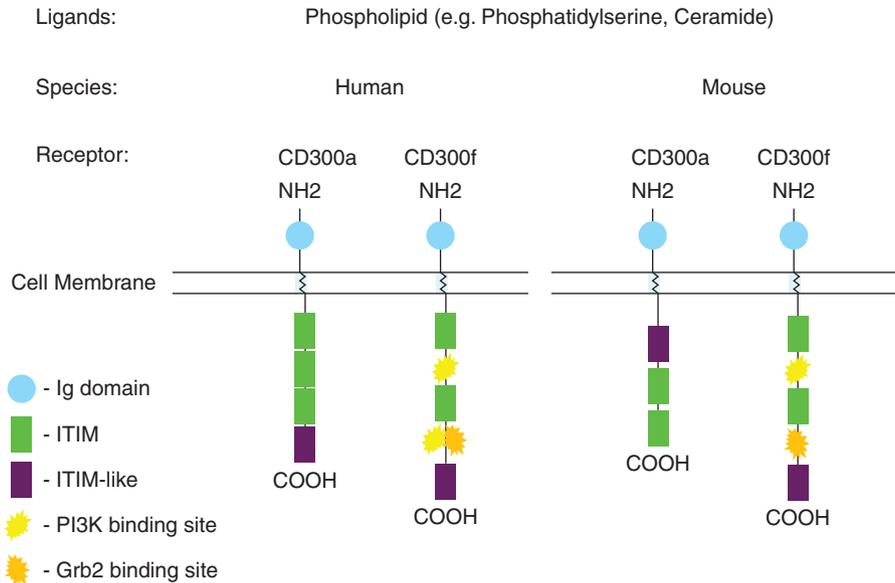CD300a, CD300f, eosinophils, inflammation, ITIM

1 | INTRODUCTION

The main task of the immune system is to eliminate pathogen invasion while preserving homeostasis. This complex mission is achieved by efficient generation, recruitment, and activation of myriad immune cells, encompassing unique activities and functions. Multiple levels of inhibitory and activation signals have evolved in the immune system such as those mediated by secreted factors (e.g., pro-inflammatory vs. anti-inflammatory cytokines), signaling intermediates (e.g., kinases vs. phosphatases), transcription factors (histone acetyltransferase vs. deacetylase), and cell surface receptors that act in concert to provide a fundamental basis for immune cell homeostasis in health and disease.[1] Among these layers of regulation, opposing signals can be generated by activating versus inhibitory cell surface receptors. The activities of these receptors are generally dictated by their intracellular signaling motifs. Inhibitory receptors are characterized by one or more ITIMs, whereas their activating counterparts have a charged residue in their transmembrane domain, which facilitates their interaction with adaptor proteins encompassing ITAMs or additional signaling motifs

such as the PI3K binding domain. The archetype ITIM sequence is composed of 6 amino acids (Ile/Val/Leu/Ser)-X-Tyr-X-X-(Leu/Val), where X denotes any amino acid.[2–5] Upon activation, the ITIMs usually undergo tyrosine phosphorylation (pTyr), often by a Src family kinase, which provides a docking site for the recruitment of cytoplasmic phosphatases having a Src homology 2 (SH2) domain such as SHP-1, -2, and/or SHIP-1, -2. Recruitment of SHP-1, -2 and/or SHIP-1, -2, results in inhibition of cell activation at a very proximal signaling stage.[2–5] The importance of inhibitory receptors in the regulation of immune cell responses has been demonstrated in various gene-targeted mice, which display increased inflammatory responses and can even develop autoimmunity.[5] Furthermore, recent advances in the field of immune oncology clearly demonstrate the vital importance of immune inhibitory receptors in antitumor immune surveillance. In this review, we will focus on the roles of CD300 family of inhibitory and activating receptors and specifically CD300a and CD300f in the regulation of eosinophil activities.

2 | CD300 RECEPTOR FAMILY

The CD300 receptor family is composed of several (i.e., 8 receptors in human and 9 in mice) type I transmembrane glycoproteins with a single IgV-like extracellular domain, which map to human chromosome 17q22–25 and chromosome 11 in mice. Although the human–mouse

Ligands: Phospholipid (e.g. Phosphatidylserine, Ceramide)

Species: Human Mouse

Receptor: CD300a CD300f CD300a CD300f

FIGURE 1 Schematic representation of human and mouse CD300a and CD300f. CD300a and CD300f contain a single extracellular Ig variable domain, a transmembrane domain, and a cytoplasmic tail that encodes for various ITIMs or ITIM-like motifs. CD300f can potentially act as a co-activating receptor since it can bind the p85α subunit of PI3K. CD300 family members such as CD300a and CD300f can bind phospholipids that are associated with cell death such as phosphatidylserine and ceramide

orthologs of the CD300 receptor family have been identified, only CD300a and CD300f have functional orthologs, whereas additional members of this family of receptors do not. Interestingly, the genetic loci of CD300 family members are one of only a few loci in the genome that is under strong positive evolutionary selection likely indicating the importance of this receptor family in immune regulation.[6]

CD300a and CD300f both have a long cytoplasmic tail containing ITIMs (Fig. 1). Thus, they are capable of eliciting negative signals by recruitment of intracellular phosphatases. In contrast to CD300a (which only possesses ITIMs or ITIM-like domains), CD300f has 2 ITIM motifs, 1 immunoreceptor tyrosine-base switch motif, 2 p85α PI3K-binding motifs (Y236 and Y263), and a growth factor receptor-bound protein 2 (Grb2)-binding motif. Therefore, CD300f is potentially capable of acting as a co-activating receptor rather than only inhibitory. Indeed, recent studies demonstrated that CD300f can partner with SHP-1 (and likely mediate inhibition) as well as with PI3K and FcR γ chain (and induce activation). Other members of the family (CD300b, CD300c, CD300d, and CD300e) have a short cytoplasmic tail and a charged transmembrane residue and associate with adaptor proteins such as DNAX-associated proteins (DAP10, DAP12) and the FcR γ chain. A structural exception to the CD300 receptor family is CD300g, which lacks biochemical signaling motifs of stimulatory or inhibitory potential and contains and a mucin-like domain.[6,7]

It is important to note that there is a real problem regarding the nomenclature of the CD300 receptor family in humans and mice. While the name in humans (CD300a–CD300h) is related to the position of the genes on chromosome 19, the names of the murine molecules may be somehow confusing. For the brevity and clarity of this review, we have used the same nomenclature for humans as mice and indicated whether we are presenting human or mouse data.

3 | DYNAMIC EXPRESSION FOR CD300 FAMILY MEMBERS

In humans and mice, CD300a and CD300f are expressed in most of the cells belonging to the myeloid lineage, including monocytes, dendritic cells (DCs), neutrophils, and eosinophils. Within the lymphoid lineage, B cells express CD300f and CD300a; and subsets of T and NK cells (especially in humans), express CD300a. In addition, CD300c is expressed by NK cells as well.[8,9] Nonetheless, the expression of CD300 receptors is highly variable, depending on the species (mouse vs. human), tissue, and cell type. Although myeloid cells express CD300 receptors under baseline conditions, the expression pattern of CD300 family members is highly dynamic and is dependent on exposure to environmental triggers, mediating cellular activation. For example, mouse bone marrow (BM)-derived DCs express high levels of CD300f, which was up-regulated by LPS and IL-10 treatment.[10] Similarly, the expression of CD300a in human neutrophils was up-regulated following stimulation with LPS and GM-CSF.[11] The expression of CD300a was also rapidly increased on the cell surface of human basophils following activation of FcεR1 and down-regulated in human plasmacytoid DCs following TLR7 and TLR9 stimulation.[12,13] Adding to this complexity is the finding that CD300 family members can appear in a soluble form as well. For example, cleavage of CD300b from mouse neutrophils has been shown to amplify inflammation in a lethal model of sepsis.[14] Collectively, the precise expression pattern of CD300 receptors should be interpreted cautiously. This is specifically true when comparing reports using different antibodies that may display cross-reactivity and recognize additional members of the CD300 family that share similar extracellular domains such as CD300a and CD300c.[15]

4 | ACTIVATION OF CD300 RECEPTORS AND THEIR PUTATIVE LIGANDS

CD300-Fc chimeric proteins are often used as a tool to discover ligands for the CD300 family members, but still little knowledge is available regarding these possible ligands. CD300a, CD300b, CD300c, and CD300f bind to phospholipids such as phosphatidylserine (PS), phosphatidylethanolamine, and ceramide, which are associated with cell death. For example, PS is exposed in the outer plasma membrane at early stages of apoptosis, serving as an "eat-me" signal for macrophages. Indeed, macrophages lacking CD300 receptors such as CD300b, CD300f, and CD300a display defects in clearance of apoptotic cells.[7,16–20] The ability of CD300b to directly bind phospholipids is still under debate as additional ligands, which may facilitate its binding to PS have been proposed such as T cell immunoglobulin mucin domain (TIM)-1.[21] Ligands for CD300 receptors are not confined to settings of cell death as murine norovirus was identified to bind murine CD300f and CD300d and this interaction dictates permissive infection.[22] Furthermore, human and mouse CD300b can bind LPS and regulate TLR4-induced responses in myeloid cells.[23]

Interestingly, CD300 family of receptors can interact with each other to form homo and heterodimers. For example, human CD300b acts as a modifier of CD300c signaling and CD300c is capable of interacting with other CD300 family receptors including CD300a, CD300f, and CD300e but no other Ig-superfamily receptors.[24,25] Thus, in addition to their diversity in ligand recognition, CD300 family receptors may elicit different signaling strengths dependent on the expression of additional CD300 family members.

5 | EXPRESSION OF CD300 RECEPTORS BY EOSINOPHILS

Although eosinophils likely express various members of the CD300 family members, protein expression data exists only regarding CD300a and CD300f. Using a flow cytometric approach, we have shown that mouse eosinophils express high levels of CD300f and that the levels of CD300f expression are different in eosinophils from distinct anatomic locations.[26] Thus, in mice, CD300f is expressed in low levels by eosinophil progenitors (EoPs) and by Siglec-F+/CCR3− BM eosinophils (representing immature eosinophils). However, as eosinophils mature in the BM (and become Siglec-F+/CCR3+), the expression of CD300f is increased and is maintained in peripheral blood and peritoneal eosinophils. Mouse colonic and adipose tissue eosinophils expressed markedly elevated levels of CD300f in comparison with eosinophils from the BM and blood.[27] It is important to note that at least in mice, contradicting data exists regarding the expression pattern of CD300f. We have recently shown that baseline tissue expression of CD300f (at least in the colon and adipose tissue) is nearly exclusively confined to eosinophils as other tissue resident cells such as macrophages, DCs and mast cells barely express CD300f.[27,28] In contrast to our findings, other groups have reported that CD300f in the colon is expressed by mast cells, macrophages and DCs.[29,30] These data suggest that different housing protocols and/or different exposure to food antigens

and perhaps endotoxins could regulate the expression of CD300f. We have shown that induction of colitis by dextran sodium sulfate (DSS) resulted in decreased expression of CD300f by colonic eosinophils and increased expression by monocytes/macrophages.[31] This observation implies distinct regulators for CD300f expression in different cells. In support of this notion, the expression of mouse CD300f (but not CD300a) was regulated by IL-5, which is a key factor in eosinophil biology and can directly promote eosinophil differentiation, maturation, priming, survival, and effector functions.[27] To this end, murine eosinophils from the BM, blood, peritoneal cavity, colon, and white adipose tissue of Il5Tg mice displayed markedly elevated levels of CD300f (but not CD300a) in comparison with eosinophils from wild-type mice. Furthermore, baseline expression of CD300f was decreased in BM eosinophils from Il5−/− mice. Although IL-5 regulated the expression of CD300f, CD300f deficiency did not alter eosinophil numbers in vivo.[27] It is noteworthy that IL-5 is capable of regulating the expression of additional Ig-superfamily receptors with inhibitory/activation capabilities. We have recently shown that IL-5 regulated the expression of paired immunoglobulin-like receptor (PIR)-B, which provides a key permissive signal for IL-5-induced eosinophil production by suppressing the pro-apoptotic activities of PIR-A.[32]

The expression of CD300 receptors in eosinophils is regulated by additional molecules such as type 2-associated cytokines. For example, IL-33 was capable of increasing the expression of CD300f in mouse eosinophils (and mast cells), whereas IL-4 increased the expression of CD300f in BM-derived mouse macrophages.[33,34] Consistent with the ability of type 2 cytokines to increase the expression of CD300f in eosinophils and macrophages, eosinophils, and macrophages that were isolated from the lungs of asthmatic mice display a specific increase in CD300f expression in macrophages and eosinophils.[33] Furthermore, assessment of CD300f expression on the surface of peripheral blood monocytes and eosinophils that were obtained from healthy and atopic individuals revealed increased expression of CD300f in comparison with healthy individuals.[33] Increased expression of CD300a (in comparison with peripheral blood expression) was also reported in nasal polyps of patients undergoing endoscopic nasal polypectomy.[35]

6 | ACTIVATION OF EOSINOPHILS BY IL-5 IS REGULATED BY CD300f AND CD300a

Eosinophils differentiate under the regulation of the common β chain cytokines IL-5, IL-3, and GM-CSF. Though IL-5 was originally described as a growth factor for B cell activation and Ig secretion, the biologic effects of IL-5 are best characterized and likely most relevant and significant on eosinophils.[36,37] IL-5, which is produced mainly by Th2 cells and type 2 innate lymphoid cells, enhances differentiation and proliferation of EoPs and is responsible for eosinophil expansion, release from the BM into the peripheral circulation, activation and survival in various disease settings.[38,39] The ability of IL-5 to increase the expression of CD300f raised the possibility that CD300f can modulate IL-5 receptor signaling. Indeed, when the IL-5-responsive mouse I.29 B cell line was transfected with CD300f and stimulated by
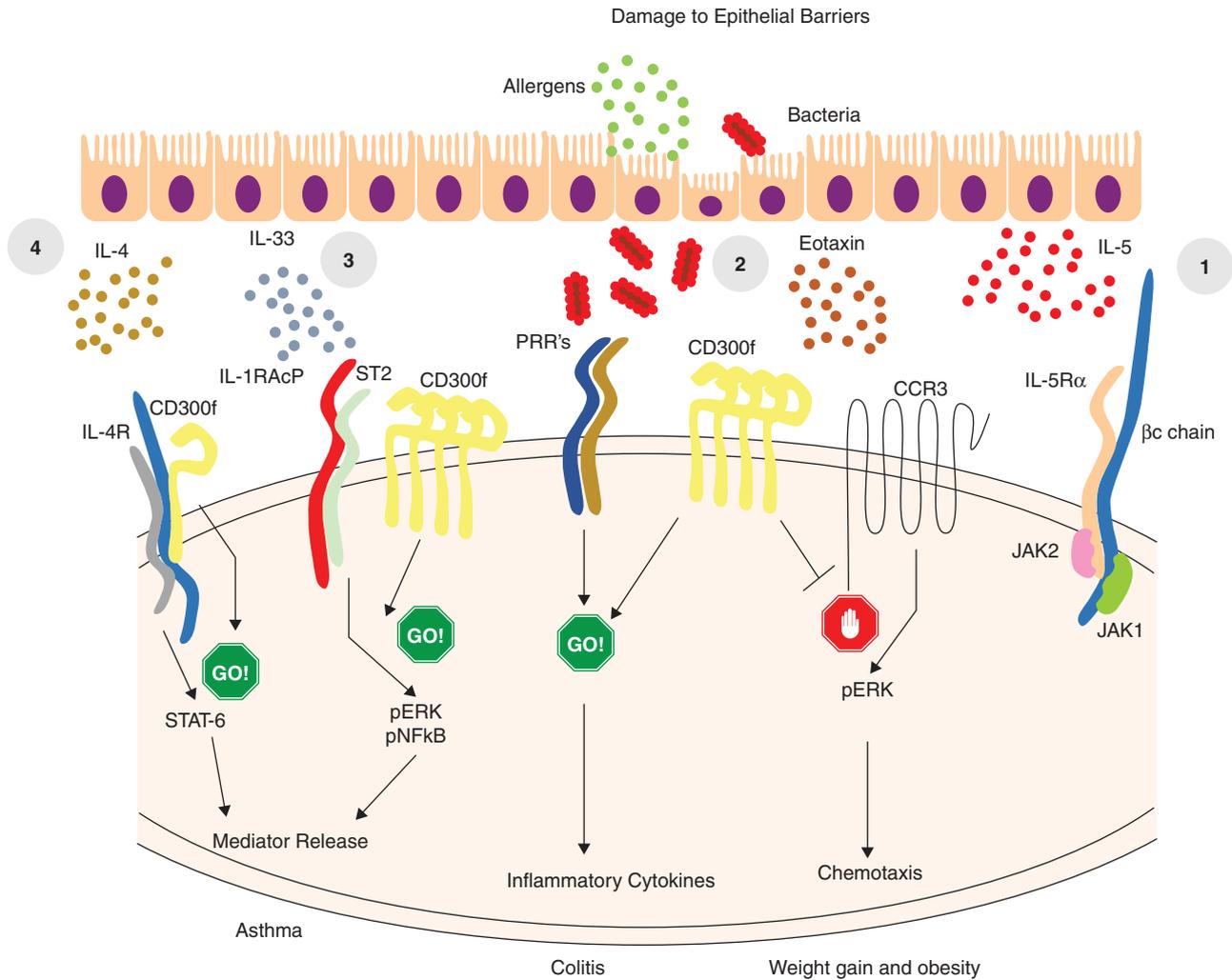
FIGURE 2 **CD300f regulated key signaling checkpoints in eosinophils**. IL-5 signaling through the IL-5Rα and βc chains regulates the baseline expression of CD300f in the bone marrow and peripheral organs. IL-5 "primes" eosinophils to respond more potently to eotaxin stimulation by potentiating CCR3-dependent signaling. In these settings, CD300f acts as a negative regulator of CCR3 by suppressing ERK activation and subsequent migration into the gastrointestinal tract and adipose tissue (1). In cases of damage to the mucosal epithelial cell barrier such as the one present in the colon, eosinophils are recruited and exposed to luminal antigens including bacterial antigens that are recognized by pattern recognition receptors (PRRs). In these settings, CD300f coactivates PRR-dependent production of pro-inflammatory cytokines, which ultimately leads to colitis (2). Additional damage to epithelial barriers can be induced by exposure to allergens, which induce the release of the type 2-associated cytokines IL-33 and IL-4. IL-33 activates eosinophils to induce the expression of CD300f, which is colocalized to the ST2–IL-1RAcP receptor complex and provides coactivating signals to amplify ERK and NFkB signaling leading to the production of pro-inflammatory cytokines (3). In addition, CD300f serves as a unique coreceptor for IL-4Rα and is physically associated with the IL-4Rα chain. CD300f amplifies IL-4-induced STAT6 signaling in eosinophils (and additional cells) resulting in optimal chemokine release and subsequent regulation of the inflammation, which accompanies asthma (4)

IL-5, PI3K was recruited to the cytoplasmic tail of CD300f, inducing downstream signal transduction which decreased ERK1/2 phosphorylation when compared to empty vector transfected cells. Furthermore, generation of *Il-5^Tg/Cd300f^−/−* mice resulted in specific and marked recruitment of eosinophils (and macrophages) into the white and brown adipose tissue and significantly elevated of IL-4 expression in eosinophils (Fig. 2).[27]

Regulation of eosinophil and macrophage accumulation in the adipose tissue of mice was of specific interest due to the newly defined functions of these cells in weight gain and the metabolic syndrome.[40] Under lean conditions, adipose tissue macrophages are activated by the Th2 cytokines IL-4 and IL-13 secreted from eosinophils and acquire an alternatively activated phenotype that mediates the secretion of anti-inflammatory cytokines (e.g., IL-10), and catecholamines, which utilize oxidative metabolism to maintain adipose tissue homeostasis.[41] In contrast, obesity is associated with a chronic state of low-grade inflammation with the presence of pro-inflammatory mediators (e.g., IL-6, TNF-α, CCL2, and iNOS) that are secreted by classically activated macrophages and induce adipocyte apoptosis and insulin resistance.[41] Since eosinophils are a major source for IL-4 in the adipose tissue,[42] eosinophil-deficient mice display increased weight gain and glucose intolerance, whereas *Il5^Tg*

mice show decreased weight gain and glucose intolerance. Remarkably, *Il5^Tg/Cd300f^−/−* mice displayed increased IL-4 expression in the adipose tissue, which resulted with increased proliferation of monocytes and macrophages and augmented differentiation into an alternatively activated phenotype, as assessed by various markers such as arginase 1. Subsequently, *Il5Tg/Cd300f^−/−* were protected from diet-induced weight gain and glucose intolerance to a larger extent than *Il5Tg/Cd300f^+/+* mice.[27] Thus, CD300f is a negative regulator of IL-5-induced eosinophil accumulation and IL-4 production in the adipose tissue. Directly related, *Cd300f^−/−* mice (even in the absence of the IL-5 transgene) displayed age-related accumulation of eosinophils and macrophages in the adipose tissue and showed decreased adipose tissue weight, which was associated with decreased diet-induced weight gain and insulin resistance.[27] The role of CD300f in the regulation of human eosinophil activities in response to IL-5 and in the adipose tissue remain to be defined.

Although CD300f was found to regulate eosinophil recruitment and activation that is primed and triggered by IL-5, it appears that CD300f does not have a significant role in additional IL-5-induced responses in eosinophils such as maturation, differentiation and survival, at least in mice. In contrast to CD300f, CD300a was shown to negatively regulate IL-5-induced human peripheral blood eosinophil survival.[43] Antibody-mediated cross-linking of CD300a (and thus artificial activation of CD300a signaling) on the surface of human peripheral blood eosinophils resulted in accelerated eosinophil apoptosis due to suppression of survival signals that were mediated by IL-5 or GM-CSF. CD300a cross-linking resulted in increased pTyr of the cytoplasmic tail followed by recruitment of SHP-1, which in turn inhibited JAK2 and MAP kinase phosphorylation.[43] Collectively, these data demonstrate that CD300a and CD300f can act as specific regulators of distinct IL-5-induced eosinophil responses.

7 | CD300a AND CD300f REGULATE EOSINOPHIL CHEMOTAXIS

Accumulation of eosinophils in peripheral organs under steady state and/or under settings of inflammation is predominantly regulated by the eotaxin family of chemokines consisting of eotaxin-1/CCL11, −2/CCL24, and −3/CCL26, which encode for CC chemokines with eosinophil-selective chemoattractant activity.[44–47] Though the mouse genome contains only *Ccl11* and *Ccl24*, the biologic activity of mouse and human eotaxin-family member is remarkably conserved and is characterized by their relative selectivity for eosinophils and CCR3, and their regulation by IL-4 and IL-13 signaling.[48]

CCR3, the eotaxin-family receptor, is a seven-transmembrane spanning G-protein-coupled receptor, primarily expressed by eosinophils.[49–51] Upon binding to its cognate ligands CCR3 recruits the kinases Hck and Gardner-Rasheed feline sarcoma viral oncogene homolog (Fgr), which presumably activate donwnstream-signaling intermediates including p38, Ras:MEK:ERK, and PI3K. CCR3 interacts with multiple ligands including MCP-2, -3, -4, RANTES, and MIP-5; however, the only ligands that exclusively signal through CCR3

are the eotaxin chemokines, accounting for the cellular selectivity of eotaxins toward eosinophils.[52] Supporting this, studies using eotaxin-1, eotaxin-2 deficient mice or eotaxin-1/-2 deficient mice have provided evidence that eotaxin-1 regulates IL-13-induced lung tissue eosinophilia, eotaxin-2 regulates airway (luminal) eosinophilia, and that both chemokines via CCR3 provide critical signals for allergen and IL-13-elicited responses.[53–55] In fact, CCR3 appears to be the dominant eosinophil chemokine receptor as suggested by its high expression level in eosinophils and the ability of CCR3/eotaxin antagonist reagents to block eosinophil recruitment.[52,56–58]

Substantial pre-clinical evidence supports a role for eotaxins in human allergic disease. Several studies have reported increased baseline levels of eotaxin-1 in the bronchoalveolar lavage fluid (BALF) of asthmatics compared with control individuals.[59,60] Following allergen challenge, eotaxin-1 is induced early (6 h) and correlates with early eosinophil recruitment; in contrast, eotaxin-2 correlates with eosinophil accumulation at 24 h.[61,62] Furthermore, analysis of single nucleotide polymorphisms in the eotaxin genes showed association with asthma susceptibility and lung function,[63–65] providing additional rationale to the importance of the CCR3:eotaxin axis in human asthma. Thus, endogenous mechanisms that regulate the eotaxin:CCR3 axis, may have significant implications in regulating the migration of eosinophils into the tissue. The importance of ITIM-bearing receptors in maintaining baseline eosinophils by regulating CCR3 signaling are nicely demonstrated by the study of gene targeted mice lacking such receptors. For example, mice lacking PIR-B, display increased eosinophil levels in their gastrointestinal tract, including the esophagus, a tissue that is usually devoid of eosinophils. Increased eosinophilia was shown to be due to negative regulation of CCR3 signaling in response to eotaxin-1 and -2. Similarly, *Cd300f^−/−* mice (but not *Cd300a^−/−* mice) displayed increased eosinophil levels in their peritoneal cavity and gastrointestinal tract. In vitro, CD300f was found to inhibit eosinophil chemotaxis towards eotaxin-1 and eotaxin-2 in a ligand-binding (i.e., PS)-dependent manner. Increased chemotaxis of eosinophils from *Cd300f^−/−* mice was accompanied with increased eotaxin-induced calcium influx and increased ERK-1/2 but not p38 phosphorylation (Fig. 2). The inhibitory role of CD300f regulating eosinophil migration had physiologic outcomes in experimental asthma. In these disease settings, eosinophil accumulation in the lungs was increased following treatment with CD300f-Fc chimeric protein which competes with cellular-expressed CD300f for ligand binding, thus decreases CD300f signaling.[26]

Comparable to CD300f, CD300a was shown to negatively regulate human peripheral blood eosinophil migration toward eotaxin as well.[43] Indeed, crosslinking of CD300a on isolated human peripheral blood eosinophils, using anti-CD300a antibody, decreased chemotaxis of eosinophils toward eotaxin-1. This activation of CD300a also resulted in inhibition of both p38 and ERK phosphorylation but had no effect on the eotaxin-induced calcium influx.[43] Furthermore, bispecific antibodies targeting CD300a and CCR3 on mouse eosinophils were capable of reversing the established asthma by suppressing eosinophil activities.[66] Despite the fact that in the latter experimental settings, CCR3 was used as a cell specific target, in order to specifically "activate" the "negative" signal elicited by CD300a in eosinophils,

it is likely that this antibody suppressed eosinophil chemotaxis as well.

8 | REGULATION OF EOSINOPHIL RESPONSES TOWARD IL-33, IL-4, AND IL-13 BY CD300 FAMILY MEMBERS

IL-33 is a member of the IL-1 cytokine family that activates various immune cells including mast cells, DCs, and eosinophils. Upon interaction with ST2 and IL-1 receptor accessory protein, it drives polarization of an immune response that culminates in the generation of IL-4- and IL-13-producing T cells. Cooperatively, these cytokines govern host defense against nematodes, and allergic diseases, such as asthma.[67] While IL-4, IL-13, and IL-33 (also termed type 2-associated cytokines) have pleotropic activities in the immune system via their ability to stimulate a plethora of cells, they can all bind and activate eosinophils as well as additional myeloid cells.[68] Given the fundamental activities that these cytokines play in allergic settings, receptors that may act to amplify or suppress ST2 and IL-4Rα signaling and subsequent IL-33/IL-4/IL-13-induced responses have great importance.

BM-derived mouse eosinophils showed a rapid, time-, and concentration-dependent increase in CD300f levels when incubated with IL-33 (but not IL-4, IL-13, IL-25, IL-1β, and TNF-α). This finding was corroborated in vivo as well as intraperitoneal administration of IL-33 up-regulated the expression of CD300f on eosinophils. Interestingly, while in vitro IL-33 did not increase the expression of CD300f in macrophages, in vivo IL-33 administration caused a significant increase in CD300f expression on macrophages and neutrophils as well.[34] These in vivo findings may be explained, at least in part by the fact that IL-4 regulated the expression of CD300f in macrophages and was capable of up-regulating CD300f expression on macrophages in vitro and in vivo.[33]

Subsequent analyses have shown that CD300f co-localized to ST2 following IL-33 stimulation and was required for IL-33-induced NFκB and p38 phosphorylation in mouse eosinophils. Furthermore, $Cd300f^{-/-}$ eosinophils stimulated with IL-33 exhibited markedly decreased secretion of IL-6, IL-4, and IL-13. Consistently, Th2 cytokine (e.g., IL-5, IL-13) and chemokine (e.g., CCL11 and CCL24) secretion was markedly attenuated in IL-33-treated $Cd300f^{-/-}$ mice (Fig. 2). Furthermore, these mice displayed reduced infiltration of mast cells, macrophages, neutrophils, and B cells, indicating that CD300f was required for IL-33-induced peritoneal inflammation.[34] CD300f was also found to physically associate with IL-4Rα and co localized with IL-4Rα on macrophages.[33] In fact, IL-4- (and IL-13-) induced mediator release and STAT6 phosphorylation was impaired in numerous myeloid cells (including eosinophils) obtained from $Cd300f^{-/-}$ mice (Fig. 2). Furthermore, CD300f activation by antibody-mediated cross-linking significantly amplified IL-4-induced mediator synthesis and release. Consistent with impaired responses toward IL-4, $Cd300f^{-/-}$ mice exhibited decreased secretion of CCL17, CCL24 and total cell infiltration in the BALF following administration of IL-4. Consistent with key roles for CD300f in the regulation of IL-33-, IL-4-, and IL-33-induced responses, CD300f was found to play a key role in asthma as well. Induction of

experimental asthma in $Cd300f^{-/-}$ mice by repetitive intranasal administrations of $Aspergilus fumigatus$, resulted in decreased serum IgE levels and attenuated levels of the IL-4/IL-13-associated chemokines CCL17 and CCL22 in the BALF in comparison with allergen-challenged wild type mice. Furthermore, allergen-challenged $Cd300f^{-/-}$ mice exhibited lower BALF eosinophil, macrophage, neutrophil, and lymphocyte cell count, confirming a decreased inflammation state. Of note, decreased IgE production in the experimental model could also result from regulation of antibody production by CD300f from B cells since splenic B cells activated by IL-4 and CD40 exhibited decreased cell proliferation and IgE secretion in the absence of $Cd300f$.[33]

9 | CD300F REGULATES INNATE IMMUNE RESPONSES OF EOSINOPHILS IN COLITIS

RNA sequencing of illeal biopsies obtained from pediatric Crohn's disease patients demonstrate increased levels of $Cd300a$, $Cd300b$, $Cd300c$, $Cd300e$, and $Cd300f$.[31] Furthermore, expression levels of the aforementioned receptors were positively correlated with the expression levels of the mucosal inflammatory marker $S100a8$ and with the existence of deep ulcers upon colonoscopy. Increased expression of $Cd300f$, but not other CD300 receptor members, was also documented in active ulcerative colitis (UC) patients, while remitted UC patients have similar $Cd300f$ expression as healthy individuals. Importantly, eosinophils in the colon of UC patients express higher levels of CD300f in comparison with neutrophils and mononuclear phagocytes.[28]

Several studies used $Cd300f^{-/-}$ mice in the DSS-induced colitis model in order to examine the role of CD300f in colitis. We have shown that DSS-treated $Cd300f^{-/-}$ mice exhibit attenuated disease activity and histopathology in comparison with DSS-treated wild type mice.[28] Decreased disease activity in $Cd300f^{-/-}$ mice was accompanied with reduced inflammatory cell infiltration and nearly abolished production of pro-inflammatory cytokines in the colon. Importantly, genetic deletion of $Cd300a$ had no effect on DSS-colitis progression. By using mixed BM chimeric mice which harbor a specific deletion of $Cd300f$ only in eosinophils, we were able to show that CD300f expression by eosinophils was required for colitis development. The regulatory role of CD300f in innate immune response by eosinophils was further validated in vitro where heat-inactivated $Escherichia coli$ activation of BM derived $Cd300f^{-/-}$ eosinophils resulted in decreased cytokine secretion and phosphorylation of pro-inflammatory cell signaling molecules.[28] In contrast to these findings, two other studies assessed the roles of CD300f in colitis and reported exacerbated colitis in $Cd300f^{-/-}$ mice compared with wild-type mice.[30,69] Interestingly, these latter studies reported distinct inhibitory activities for CD300f. Matsukawa et al.[69] demonstrate that interactions between CD300f and ceramide inhibited ATP-stimulated activation of colonic mast cells, whereas Lee et al.,[30] demonstrate a failure of $Cd300f^{-/-}$ mice to resolve colonic inflammation, due to defects in DC function associated with abnormal apoptotic cell build-up in the gut. Thus, CD300f-deficient DCs display hyperactive phagocytosis of apoptotic cells, which stimulates excessive TNF-α secretion and

subsequently prolonged colonic inflammation. Thus, the role of CD300f in colonic inflammation is currently unclear especially since all three studies used similar induction protocols (i.e., DSS) for establishing the experimental disease. While these data argue for different regulatory roles for CD300f, the different results could be explained by various factors including technical aspects such as institutional differences (e.g., dietary source and composition, age of mice) and variability in *Cd300f*-deficient mouse strains. Indeed, all three studies used different mouse-strain of *Cd300f*$^{−/−}$ created in different laboratories. Moreover, different expression patterns of CD300f were noted in the different studies. We demonstrated high expression levels for CD300f in eosinophils and neutrophils in the colon and almost no expression by colonic mast cells, DCs, and macrophages, whereas Matsukawa et al. reported CD300f expression by colonic mast cells, macrophages, neutrophils, and eosinophils but not by CD11b$^+$/CD11c$^+$ cells (presumably being DCs). Adding to this complexity, Lee et al. demonstrated CD300f expression by DCs as well. Since the expression of CD300f and of additional CD300 family members can be regulated by various environmental factors including endotoxin,[33,34] different microbiota communities or dietary source between the three studies may modulate CD300f expression in the gut. Finally, but not least important, the overall signaling capability of CD300f family members is dependent on their ability to form homo- and heterodimers. In fact, recent data demonstrate that distinct combinations of CD300 receptors in a signaling complex differentially regulate the signaling outcome. Thus, differences in expression of additional CD300 family members may directly impact the strength and possibly, the direction (e.g., inhibition vs. coactivation) of the signal.

10 | CONCLUDING REMARKS

CD300 family receptors are emerging as important regulators of various cellular processes including efferocytosis, immune regulation, and cytokine signaling. Furthermore, assessment of various experimental disease models in mice using gene-targeted mice of functional blockade by ligand competition demonstrated the involvement of CD300 family members in multiple pathologies, including autoimmune disorders,[70,71] nerve regeneration,[72] asthma,[33] colitis,[28,30,69] sepsis,[14,23,73] viral infections,[22,74] acute kidney injury, and brain damage.[21,75] Among their activities, CD300 receptors and especially CD300a and CD300f have been shown to regulate eosinophil functions in response to diverse stimuli and in response to key cytokines and chemokines in eosinophil biology such as IL-5, eotaxins, and type 2-associated cytokines. Transition of these findings in the future into human eosinophils and assessing the roles of additional CD300 family members in eosinophils may provide new pharmacologic tools to target eosinophils in multiple disease settings.

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