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Review Article

# The CD300 molecules: an emerging family of regulators of the immune system

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**The CD300 family of molecules modulates a broad and diverse array of immune cell processes via their paired activating and inhibitory receptor functions. The description that CD300 molecules are able to recognize lipids, such as extracellular ceramide, phosphatidylserine, and phosphatidylethanolamine, that are exposed on the outer leaflet of the plasma membrane of dead and activated cells has opened a new field of research. Through their binding to lipids and other ligands, this family of receptors is poised to have a significant role in complex biological processes and in the host response to severe pathological conditions. Indeed, published data have demonstrated their participation in the pathogenesis of several disease states. Moreover, this family of receptors has great potential as targets for diagnosis and therapeutic purposes in infectious diseases, allergies, cancer, and other pathological situations. For instance, one member of the family, CD300a, has been studied as a possible biomarker. Here, a review is provided on the cellular distribution of the human and mouse families of receptors, the stimuli that regulate their expression, their ability to tune leukocyte function and immune responses, their signaling pathways, ligand recognition, and their clinical relevance. (*Blood*. 2013;121(11):1951-1960)**

## Introduction

In order to provide an adequate response that allows the elimination of insults while preserving self, the immune system is tightly regulated by a balance between activating and inhibitory signals. Multiple mechanisms exist to accomplish this task, including the expression of activating and inhibitory receptors by immune cells.[1-5] In general, the inhibitory receptors carry immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic tail,[2,4] whereas their activating counterparts have a charged residue in their transmembrane segments that facilitates the interaction with adaptor proteins carrying immunoreceptor tyrosine-based activating motifs or phosphatidylinositol 3-kinase (PI3K) binding motif (YxxM).[2,6]

The human CD300 multigene family has 7 members located on chromosome 17.[7-9] They were named alphabetically according to the order of their location in the chromosome. The mouse counterparts, which were reported as dendritic cell (DC)–derived Ig-like receptor (DIgR),[10] CMRF-35–like molecules (CLM),[11] leukocyte mono–Ig-like receptor (LMIR),[12] and myeloid-associated Ig-like receptor (MAIR),[13] are encoded by 9 genes located on mouse chromosome 11, the synthenic region of human chromosome 17.[9] The human-mouse CD300 orthologs have been identified by phylogenetic analysis and by their gene organization within the complex.[9] However, except for the 2 inhibitory receptors (ie, CD300a and CD300f), all the others are not perfect functional orthologs. For the purpose of clarity, and based on the published literature, this review will use the CD nomenclature for the human molecules and for the 2 murine ITIM-containing receptors. For the rest of the mouse CD300 molecules, the nomenclature is still very confusing, and in this review, sometimes a combination of names will be used when referring to the same receptor. Table 1 and Table 2 summarize the nomenclature these receptors have received since their discoveries. In Figure 1, the genomic organization of the human and mouse CD300 complexes is represented according to the latest drafts provided by the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/gene) and the Mouse Genomic Informatics (MGI; http://www.informatics.jax.org). For the murine genes, the CD nomenclature has not been used for all the genes in Figure 1, but it is shown in Table 2.

CD300 receptors are type I transmembrane proteins with a single IgV-like extracellular domain containing 2 disulfide bonds.[9,14,15] Only CD300a and CD300f have long cytoplasmic tails with ITIMs, whereas the other members have a short cytoplasmic tail and a charged transmembrane residue and associate with adaptor proteins such as DNAX associated protein (DAP)12, DAP10, and the Fc receptor (FcR)γ chain (FcεRIγ)[9] (Figure 2). One exception is CD300g, which is encoded by a gene mapped at some distance from the complex, lacks structural motifs suggestive of stimulatory or inhibitory potential, and in addition to the IgV-like domain, has a mucin-like domain.[16,17] CD300g is expressed in the vascular endothelial cells of high endothelial venules of lymph nodes and supports lymphocyte rolling via the mucin-like domain, mediates lymphocyte binding through its Ig domain, and promotes lymphocyte transendothelial migration in vitro.[16-18]

## Distribution, mechanisms of signaling, and modulation of immune functions

CD300 family members have the potential to form homo- and heterodimers, which is dependent on their immunoglobulin (Ig) domains. This implies that in addition to the signal that originates from each single receptor, the formation of heterocomplexes adds another degree of complexity in the signaling pathways emanating

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**Table 1. Human CD300 family members**

| CD nomenclature | Alternative names | Human Genome Organization Gene Nomenclature Committee |
|---|---|---|
| CD300a | CMRF-35H, IRp60, IRC1, IRC2 | CD300A |
| CD300b | CD300lb, IREM-3 | CD300LB |
| CD300c | CMRF-35A | CD300C |
| CD300d | CD300ld | CD300LD |
| CD300e | IREM-2 | CD300E |
| CD300f | CD300lf, IREM-1, IgSF13 | CD300LF |
| CD300g | CD300lg, nepmucin | CD300LG |

**Table 2. Mouse CD300 family members**

| Names used in the literature* | NCBI† and MGI† nomenclature |
|---|---|
| CLM-8, LMIR-1, MAIR-I | CD300a |
| CLM-7, LMIR-5, CD300b, mIREM3 | CD300lb |
| CLM-6 | CD300c |
| CLM-5, LMIR-4, MAIR-IV | CD300ld |
| CLM-4, LMIR-2, MAIR-II, DIgR1, CD300d | AF251705 |
| CLM-3, LMIR-7 | CD300lh |
| CLM-2 | CD300e |
| CLM-1, DIgR2, LMIR-3, MAIR-V | CD300lf |
| CLM-9, nepmucin | CD300lg |

*Names based on the published literature and used in the reference list of this review.

†http://www.ncbi.nlm.nih.gov/gene; http://www.informatics.jax.org.

from this family of receptors.[19,20] Here, both the human and mouse ITIM-containing CD300a and CD300f receptors will be jointly reviewed because they can be considered as functional orthologs. However, description of non–ITIM-containing CD300 receptors from humans and mice will be discussed separately.

### ITIM-containing CD300 receptors

Transcripts encoding human CD300a are detected in myeloid and lymphoid cells.[21-26] Nevertheless, the cell surface expression of CD300a has been somewhat difficult to determine due to a lack of monoclonal antibodies (mAbs) able to distinguish between CD300a and CD300c.[23,25,27,28] Human CD300a is expressed in all natural killer (NK) cells[22,25] and in subsets of T and B cells.[27-31] On $CD4^+$ T cells, the expression of CD300a is associated with T helper 1 (Th1) cells that are more polyfunctional and after stimulation upregulate the transcription factor eomesodermin.[28,29] On $CD8^+$ T cells, CD300a expression is mostly associated with effector functions.[31] Naive B cells express low levels of CD300a, whereas memory B cells and plasma cells express variable levels.[30] CD300a is also expressed on the surface of monocytes, plasmacytoid dendritic cells (pDCs) and myeloid dendritic cells (mDCs), granulocytes, and cord blood–derived mast cells.[24,32-36] For $CD4^+$ T cells, stimulation with anti-CD3 plus anti-CD28 mAbs and Th1 differentiation conditions upregulated cell surface expression of CD300a, whereas transforming growth factor β1 (TGF-β1) exhibited a negative regulatory effect.[27,29] Toll-like receptor (TLR)9 stimulation increased the cell surface expression of CD300a on human memory B cells, whereas interleukin (IL)-4 and TGF-β1 were negative regulators.[30] lipopolysaccharide (LPS), interferon (IFN)-γ, IFN-α, and hypoxia conditions regulated the expression of CD300a in monocytes.[21,37] In neutrophils, LPS and granulocyte macrophage–colony-stimulating factor (GM-CSF) treatment caused a rapid translocation of an intracellular pool of CD300a to the cell surface.[32] It is quite possible that an intracellular pool of CD300a also exists in human basophils, which is translocated to the cell surface upon their stimulation through the FcεRI in just 1 to 3 minutes.[36] In pDCs, IFN-α production in response to TLR7 and TLR9 stimulation downregulated the expression of CD300a,[24] and the eosinophil-derived major basic protein and eosinophil-derived neurotoxin downregulated it on cord blood–derived mast cells.[33]

Transcripts encoding mouse CD300a are also found in lymphoid and myeloid cells. On the cell surface, mouse CD300a is expressed on the majority of myeloid cells, and on subsets of B cells, with higher expression on marginal zone B cells.[12,13] However, unlike human CD300a, it is not detected on the surface of unstimulated NK and T cells, and stimulation with IL-12 resulted in low expression of the receptor on NK cells.[13]

Human CD300a has 4 tyrosine residues in its intracellular tail. Three of those tyrosines are within consensus sequences for classical or canonical ITIMs (I/V/LxYxxL/V), and the fourth is part of a nonclassical or permissive ITIM (I/V/L/S/TxYxxL/V/I) (Figure 2).[22,38] The concept of nonclassical or permissive ITIM includes the previously described immunoreceptor tyrosine-based switch motif (TxYxxV/I).[38] The cytoplasmic tail of mouse CD300a possesses 2 classical ITIMs, and a third tyrosine is within a tyrosine-based sorting motif.[12,13] Tyrosine phosphorylation of the ITIMs is required for the transmission of the inhibitory signal,[25,39,40] and in CD300a transfected Jurkat cells, the Src tyrosine kinase Lck is responsible for their phosphorylation.[39] Phosphorylated ITIMs are able to recruit different phosphatases depending on the examined cell type and the method of stimulation.[12,22,33,35,40] In DT40 B cells deficient in Src homology 2 domain-containing phosphatase (SHP)-2 and SH2-containing inositol phosphatase (SHIP), CD300a was still capable of inhibiting B-cell receptor (BCR)–mediated signals. However, in cells deficient in SHP-1, the CD300a-mediated inhibition was largely abolished, indicating a dominant role for this phosphatase in CD300a-mediated signal.[39] Furthermore, only the pharmacologic inhibition of SHP-1 was able to block the inhibitory function of CD300a.[41] In a more physiological setting, SHP-1 recruitment to mouse CD300a was observed when bone marrow–derived mast cells (BMMCs) and macrophages were mixed with apoptotic cells and treated with LPS.[42,43] Apoptotic cells expose phosphatidylserine (PS) and phosphatidylethanolamine (PE), the 2 ligands of CD300a, in the outer leaflet of the plasma membrane. The dominant role of SHP-1 has been also demonstrated by knocking down SHP-1 expression in BMMCs from mice.[43]

Cross-linking of human CD300a with mAbs inhibited $Ca^{++}$ mobilization in response to stimulation mediated by BCR,[30] T-cell receptor (TCR),[28] FcεRI,[33,44,45] and FcγRIIa.[32] It also inhibited NK cell–mediated cytotoxicity[22,25]; B-cell proliferation[30]; IgE-dependent mediator release from mast cells, stem cell factor (SCF)–mediated mast cell activation, differentiation, and survival, and IgE-mediated CD63 expression in basophils[33,36,44,45]; eotaxin-induced eosinophil migration and eosinophil survival induced by IL-5 and GM-CSF[35]; FcγRIIa-mediated reactive oxygen species production by neutrophils[32]; and LPS and CpG oligodeoxynucleotides (CpG)–induced IL-8 secretion by the myelomonocytic THP-1 and U937 cell lines.[41] Cross-linking of CD300a also regulated TCR–mediated IFN-γ production[28] and type I interferon and tumor necrosis factor α (TNF-α) secretion by pDCs in response to TLR7 and TLR9 stimulation.[24] Engagement of mouse CD300a with mAbs inhibited IgE-induced mediator release from BMMCs.[13] $CD300a^{-/-}$ BMMCs and macrophages treated with LPS in the presence of apoptotic cells produced higher levels of proinflammatory cytokines, indicating that CD300a acts as an inhibitory receptor in these cell types.[43]

Human CD300f transcripts were detected in myeloid cells.[46,47] On the cell surface, CD300f is expressed on monocytes, although almost

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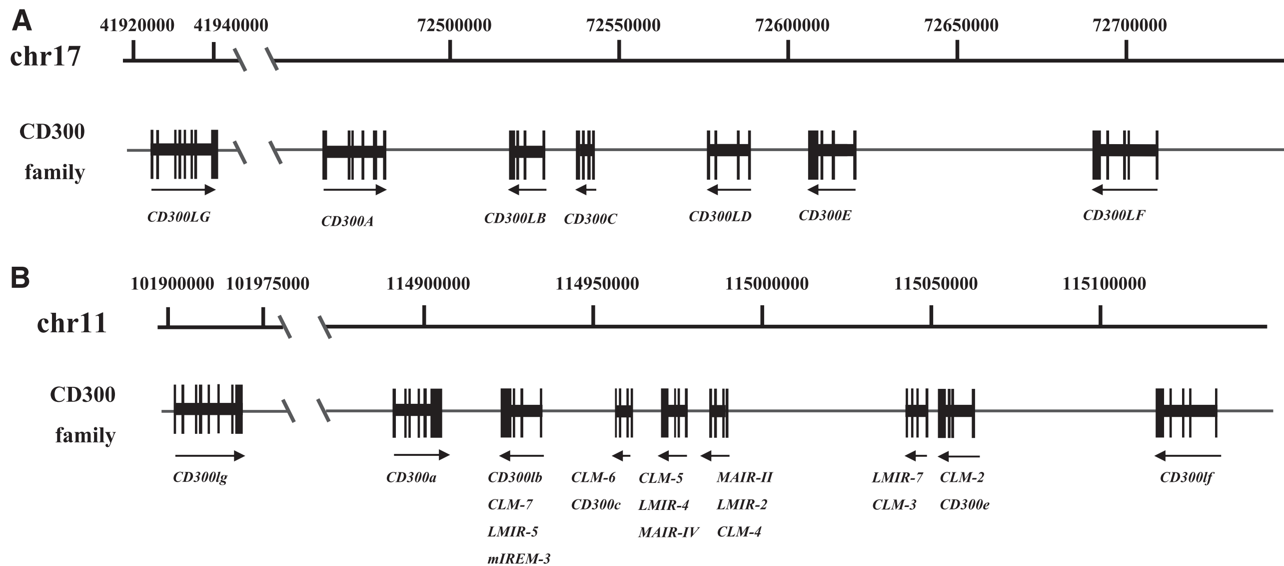


**Figure 1. Schematic diagrams showing the organization of the CD300 gene complexes in human (A) and mouse (B).** For the murine complex, the CD nomenclature according to the latest draft provided by NCBI and MGI has not been used for all the genes. For the complete CD nomenclature of the murine genes, see Table 2.

absent in monocytes from cord blood, and at low levels in circulating DCs, granulocytes, and monocyte-derived DCs.[46,48,49] Interestingly, CD300f was markedly upregulated in monocyte-derived DCs that were cultured in the presence of 1,25-dihydroxyvitamin $D_3$, which reprograms DCs to become tolerogenic, suggesting an important role for this receptor in maintaining a tolerogenic state.[50] Expression of CD300f was also found in the majority of acute myeloid leukemia (AML) samples obtained from patients.[48] Mouse CD300f transcripts are found in myeloid cells, including mast cells, and in B cells.[11,51] Bone marrow (BM)-derived DCs also express abundant levels of CD300f transcripts, which are upregulated by LPS and IL-10 treatment.[51] The cell surface expression of CD300f on neutrophils is upregulated after LPS and GM-CSF stimulation,[52] and treatment with receptor activator of nuclear factor  B (NF-κB) ligand, a cytokine that drives osteoclast formation, downregulates CD300f messenger RNA (mRNA) levels.[11]

Human CD300f has 5 tyrosine-based motifs in the intracellular tail; 2 of them fit with classical ITIMs and a third with a nonclassical ITIM. The other 2 tyrosines are within motifs reported to bind the p85α regulatory subunit of PI3K (YxxM), with one of them additionally fitting a growth factor receptor–bound protein 2 (Grb2) binding motif (YxN).[46,53] Mouse CD300f has 2 classical ITIMs, a nonclassical ITIM, a PI3K binding motif, and a Grb2 binding motif.[11] As for CD300a, ITIM tyrosine phosphorylation is required for the CD300f-mediated inhibitory signal, and phosphorylated ITIMs are able to recruit different phosphatases depending on the examined cell type.[11,46,47,53,54] The cross-linking of CD300f in THP-1 cells resulted in phosphorylation of SHP-1 and SHP-2, whereas the pharmacologic inhibition of both phosphatases was able to block CD300f-mediated signals.[41,55-57] In anti-trinitrophenyl IgE-sensitized BMMCs, coengagement of FcεRI and mouse CD300f with trinitrophenyl-bovine serum albumin and ceramide, respectively, induced the tyrosine phosphorylation of CD300f and the subsequent recruitment of SHP-1 and SHP-2, but not of SHIP.[58] Extracellular ceramide has been described as a natural ligand for mouse CD300f.[58]

Engagement of CD300f with mAbs inhibited FcεRI-mediated degranulation.[53] On THP-1 cells, CD300f inhibited the expression of IL-8 and matrix metalloproteinase 9 induced by TLR2, TLR3, TLR4, TLR9, and B-cell activation factor.[41,55,56] Overexpression of CD300f in RAW cells inhibits in vitro osteoclast formation by receptor activator of NF-κB ligand and TFG-β,[11] and coengagement of FcεRI and CD300f with mAbs in BMMCs impaired IL-6 production induced by FcεRI cross-linking alone.[54] In in vitro experiments, the cross-linking of mouse CD300f with its ligands ceramide, sphingosylphosphocholine (SPC), high-density lipoprotein, or low-density lipoprotein inhibited FcεRI-mediated β-hexosaminidase release and IL-6 production. This inhibition was not observed when CD300f$^{-/-}$ BMMCs were used in the assay.[58] Using CD300f-Fc fusion protein as a reagent to block the interaction of CD300f with its ligands, and short interfering RNA technology, it has also been shown that this receptor inhibits DC-induced T-cell proliferation in vitro. Furthermore, CD300f negatively regulates DC-initiated antigen-specific T-cell responses both in vivo and in vitro and inhibits antigen-specific Th1 and cytotoxic T lymphocyte responses in vivo.[51]

A feature of CD300f is that it also exhibits an activating potential that is more evident when CD300f is cross-linked in cells that express receptors with mutated ITIMs.[53,54] For human CD300f, both tyrosines within the 2 PI3K binding motifs recruit the p85α subunit of PI3K, and SHP-1 does not block its recruitment.[53] In addition to PI3K, CD300f also binds Grb2. The recruitment of the p85α subunit of PI3K and Grb2 to the cytoplasmic tail of mouse CD300f has been demonstrated by some authors,[54] but not by others.[11] Intriguingly, cross-linking of mouse CD300f was able to induce IL-6 production in cells expressing a CD300f construct with all the intracellular tyrosines mutated to phenylalanine,[54] an effect that was not observed with human CD300f.[53] This prompted the search for partners of mouse CD300f that are responsible for the activating signal. In fact, the FcRγ chain was shown to associate with mouse CD300f, and more importantly, it was shown that FcRγ chain–deficient BMMCs expressing CD300f with all the tyrosine residues mutated were severely impaired in their ability to produce IL-6.[54] Moreover, cross-linking of wild-type mouse CD300f enhanced IL-6 production of BMMCs stimulated by LPS, in a process dependent on the intracellular tyrosines and the FcRγ chain. On the other hand, cross-linking of wild-type mouse CD300f suppressed IL-6 cytokine production in response to other TLR agonists or SCF.[54] Thus, the mouse inhibitory receptor CD300f has a unique property to associate with FcRγ chain and thereby functions as an activating receptor in concert with TLR4 stimulation. Lastly, mouse

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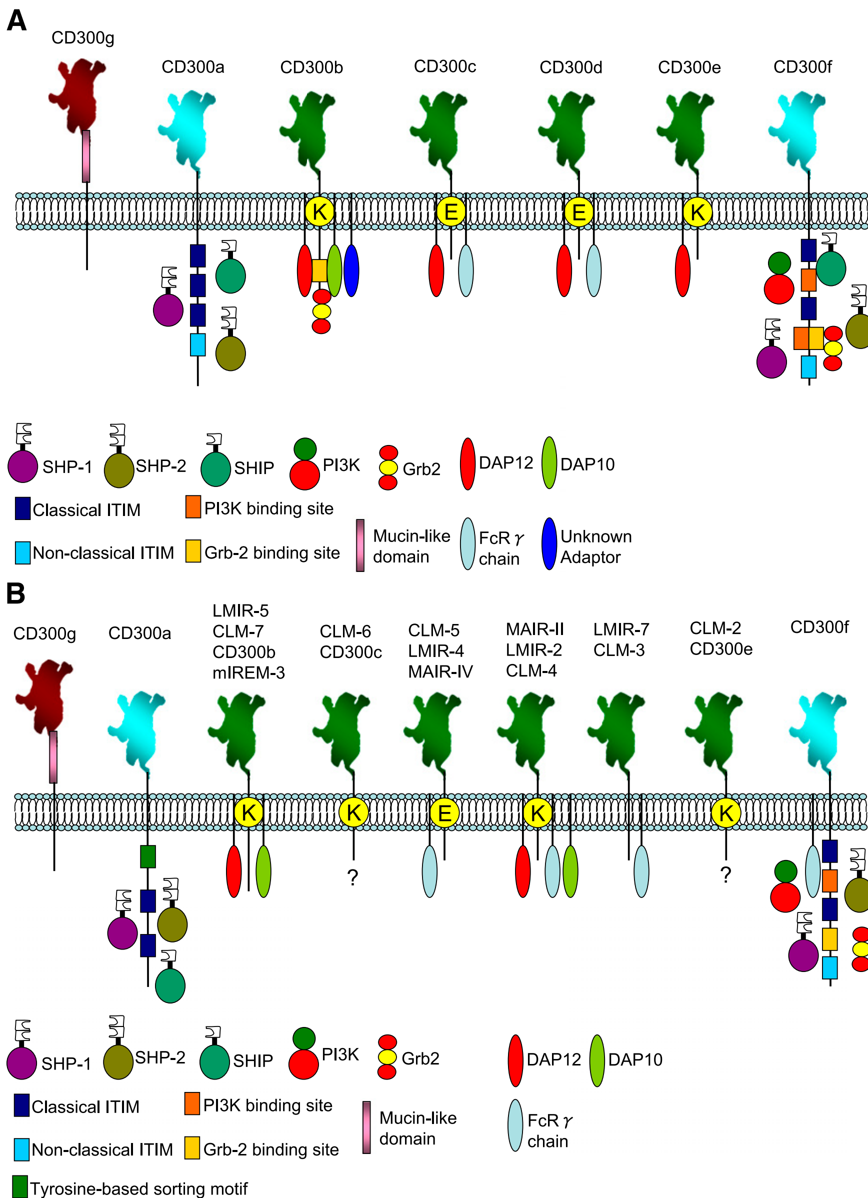


**Figure 2. Schematic representation of the CD300 receptors in human (A) and mouse (B).** The extracellular domain of the ITIM-containing receptors is colored in turquoise, and that of the non–ITIM-containing receptors is colored in green. The extracellular domain of CD300g is colored in brown. The signaling motifs of each receptor are indicated, and the interaction with phosphatases, adaptor molecules, and kinases is shown. It is important to note that some associations of CD300-activating molecules with adaptor proteins have been demonstrated only in cells transfected with complementary DNAs encoding the corresponding adaptor proteins. The question marks indicate that the signaling pathway is currently unknown. The nomenclature for the mouse receptors follows the nomenclature shown in Figure 1.

CD300f has also been shown to enhance phagocytosis of apoptotic cells when expressed in the fibroblast cell line L929, although the signaling intermediates involved in this signaling pathway are not known.[59]

An additional and intriguing aspect of mouse CD300f is its ability to mediate apoptosis on myeloid cells. Cross-linking of CD300f with mAbs mediated cell death that was dependent on the cytoplasmic tail but did not require intact ITIMs or PI3K activity. CD300f-mediated apoptosis was independent of caspase, endoplasmic reticulum stress, and autophagy.[60] More studies are required to further delineate the ability of mouse CD300f to regulate cell survival and to show if human CD300f has a similar property.

### Human non–ITIM-containing CD300 receptors

Human CD300b transcripts are detected in myelomonocytic cells and weakly in some NK and B cell lines.[61,62] All-*trans*-retinoic acid in combination with phorbol 12-myristate 13-acetate increases the expression of CD300b in THP-1 cells.[62] CD300b contains a lysine in the transmembrane segment that is required for its association with DAP10 and DAP12.[61,63] It also has a Grb2 binding motif in the cytoplasmic tail that is tyrosine phosphorylated and is capable of recruiting Grb2.[61] The phosphorylation of the Grb2 motif was observed in DAP12$^{-/-}$ but not DAP12$^{+/+}$ BMMCs transduced with CD300b.[63] Accordingly, in the presence of DAP12, the Grb2 binding motif does not mediate activation signals, and other signaling pathways (eg, those involving hexosaminidase granule release) were only activated when DAP12 was present,[61,63] suggesting that this adaptor is the main contributor to human CD300b-mediated signal. The Grb2 binding motif was sufficient for transmitting activation signals when human CD300b was expressed in the DAP12-negative RBL-2H3 cells[61] and in DAP12 or DAP10/DAP12 double-deficient BMMCs.[63] An additional unidentified adaptor has been proposed because under certain experimental conditions the absence of both DAP12 and DAP10 in combination with the mutated cytoplasmic tyrosine did not completely inhibit the activating signal, whereas it was completely abrogated when the transmembrane lysine was mutated to a glutamine.[63]

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Like CD300a, transcripts encoding CD300c are found in almost all leukocytes.[9] CD300c transcription is downregulated in pDCs in response to TLR7 and TLR9 stimulation.[24] The ligation of CD300c expressed in RBL-2H3 cells with Abs is able to deliver activating signals that are mediated by the transmembrane glutamic acid residue and by its association with the FcRγ chain.[20]

CD300d transcripts are found in the myeloid compartment. However, CD300d is not expressed on the cell surface of primary cells, suggesting a mechanism that regulates the trafficking of this receptor in human cells. It can be expressed on the cell surface of COS-7 when cotransfected with FcRγ chain. However, it is not detected on the surface of cells that normally express the FcRγ chain and have CD300d mRNA.[19] CD300d associates with other CD300 molecules and is able to decrease the cell surface expression of CD300f when both of them are expressed in COS-7 cells.[19] Differentiation of monocytes toward macrophages was accompanied with a decrease in the levels of CD300d mRNA, particularly in IL-13–driven type II macrophages.[19] The ability of CD300d to deliver activating signals is not known.[19]

CD300e is expressed on the cell surface of monocytes and circulating mDCs in vivo, although it is expressed at low levels in in vitro–derived macrophages and DCs.[64-66] It has a lysine in the transmembrane segment allowing its association with DAP12.[64] Cross-linking of CD300e with mAbs was able to induce activation signals, such as transcriptional activity of nuclear factor of activated T cells, $Ca^{++}$ mobilization, and release of reactive oxygen species in monocytes.[64,65] It also induced cytokine release and expression of activation markers and promoted survival of monocytes and circulating mDCs.[64,65] Additionally, DCs activated via CD300e exhibited a higher capacity to stimulate T cells.[65]

#### Mouse non–ITIM-containing CD300 receptors

CD300lb/LMIR-5/CLM-7/mIREM-3 transcripts are found in myeloid cells and is expressed on the surface of granulocytes, DCs, macrophages, and mast cells.[63] Neutrophils constitutively shed a soluble form of CD300lb/LMIR-5/CLM-7/mIREM-3 in vitro and in vivo, in a process mediated by matrix metalloproteinases, and TLR4 stimulation increased its release.[67] DAP12 plays the major role in delivering the activating signals, and to a lesser extent DAP10.[63,68] Engagement of the receptor with Abs induced extracellular signal-regulated kinase (ERK), p38, and Akt phosphorylation; cytokine and chemokine production; cell adhesion; histamine release; and mast cell survival.[63] The cross-linking of endogenous CD300lb/LMIR-5/CLM-7/mIREM-3 with Abs induced Syk-dependent activation of fetal liver mast cells.[63] Its engagement by T-cell Ig mucin (TIM)-1, its ligand, also induced ERK phosphorylation in CD300lb/LMIR-5/CLM-7/mIREM-3 transduced BMMCs and IL-6 production.[68]

MAIR-II/LMIR-2/CLM-4/DIgR1 transcripts are found in myeloid cells and in B cells.[10,12,13] On the cell surface, the receptor is found on BM-derived DCs, BMMCs, peritoneal macrophages, and subsets of B cells.[10,12,13] LPS treatment upregulated its expression in splenic B cells and in macrophages through a mechanism involving the enhancement of FcRγ chain expression.[13,69] MAIR-II/LMIR-2/CLM-4/DIgR1 possesses a lysine in the transmembrane region that is important for its association with adaptor proteins.[69] DAP12, DAP10, and the FcRγ chain have been shown to interact with MAIR-II/LMIR-2/CLM-4/DIgR1 when they are expressed in COS1 cells.[12] However, it associates only with endogenous DAP12 in transfected RAW cells and in splenic B cells and macrophages.[13,69] In peritoneal macrophages, it associates with endogenous DAP12 and the FcRγ chain.[69] MAIR-II/LMIR-2/CLM-4/DIgR1 mediates activation signals that are dependent on DAP12 in splenic macrophages and in transfected RAW cells,[13,69] and on both DAP12 and the FcRγ chain in peritoneal macrophages.[69] Ligation with mAbs resulted in cytokine production by MAIR-II/LMIR-2/CLM-4/DIgR1 transfected RAW cells and by peritoneal macrophages.[13,69] LPS has an additive effect on MAIR-II/LMIR-2/CLM-4/DIgR1–mediated activation signal as a result of increased cell surface expression of the receptor on peritoneal macrophages.[69] Surprisingly, MAIR-II/LMIR-2/CLM-4/DIgR1 has an inhibitory effect in B cells.[70] Like DAP12-deficient B cells, MAIR-II/LMIR-2/CLM-4/DIgR1–deficient cells showed an enhanced proliferation in response to BCR and CpG stimulation. Furthermore, expression of a chimeric receptor composed of the extracellular domain of the receptor directly coupled to DAP12 into $DAP12^{-/-}$ or MAIR-II/LMIR-2/CLM-4/$DIgR1^{-/-}$ B cells was able to suppress BCR- and CpG-mediated proliferation. The inhibitory effect was mediated by recruitment of SHP-1 to MAIR-II/LMIR-2/CLM-4/DIgR1–associated DAP12 after BCR stimulation.[70]

Transcripts encoding mouse LMIR-7/CLM-3 are detected in BMMCs, BM-derived macrophages, peritoneal macrophages, and several cell lines.[71,72] Conflicting results have been reported related to the cell surface expression of LMIR-7/CLM-3. Enomoto et al,[71] using a specific anti–CLM-3 mAb, have shown that this receptor is expressed on the surface of neutrophils, monocytes , and peritoneal macrophages, as well as BMMCs and BM-derived macrophages. On the other hand, Wu et al[72] only detected LMIR-7/CLM-3 in the endosomal/lysosomal compartments and not on the cell surface. The reason for these discrepant results is not clear, although it may be related to the techniques used to detect the expression of LMIR-7/CLM-3. This receptor associates with the FcRγ chain, although it does not possess a charged residue in the transmembrane domain.[71] Cross-linking of LMIR-7/CLM-3 on transduced BMMCs induced activation signals and cytokine and chemokine production. These activation signals were abolished when the receptor was cross-linked in FcRγ chain–deficient BMMCs.[71] Interestingly, as a result of its endosomal/lysosomal localization, it has been shown that LMIR-7/CLM-3 functions as a positive regulator of TLR9, but not of TLR4 or TLR3.[72] Results from transfected cells and knockdown experiments in peritoneal macrophages showed that LMIR-7/CLM-3 upregulates TLR9-mediated production of the proinflammatory cytokines TNF-α and IL-6 but does not affect type I IFN expression.[72] Further confirmation of LMIR-7/CLM-3–mediated effects on TLR9 signals was obtained by using macrophages derived from LMIR-7/CLM-3 transgenic mice.[72] What is more, the LMIR-7/CLM-3–mediated promotion of TLR9 production of proinflammatory cytokines was through a mechanism that involves the ubiquitination of TRAF6, a signaling mediator of TLR-triggered NF-κB and mitogen-activated protein kinase activation.[72]

CLM-5/LMIR-4/MAIR-IV transcripts are detected in myeloid cells.[52,73] On the cell surface, the receptor is expressed on granulocytes, on monocytes, on macrophages, and in BM-derived mDCs and pDCs.[52,74] Although LPS downregulated CLM-5/LMIR-4/MAIR-IV expression on neutrophils, GM-CSF increased it.[52] Notwithstanding that CLM-5/LMIR-4/MAIR-IV has a charged transmembrane residue, it seems that it is not required for its association with the FcRγ chain.[52,73,74] In fact, the interaction of CLM-5/LMIR-4/MAIR-IV with the FcRγ chain depends on the extracellular juxtamembrane region as well as its transmembrane domain.[71] The critical role of the FcRγ chain in CLM-5/LMIR-4/MAIR-IV–mediated signaling transmission was shown by the complete absence of activation signals in FcRγ chain–deficient BMMCs following ligation with Abs.[52] Lyn and Syk kinases are

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also involved in CLM-5/LMIR-4/MAIR-IV signal transmission.[52] Engagement with mAbs in BMMCs transduced with the receptor induced IL-6 production and histamine release and delivered an antiapoptotic signal. Additionally, the combination of CLM-5/LMIR-4/MAIR-IV, TLR4, and FcεRI stimulations synergistically increased cytokine production by BMMCs.[52] In neutrophils and peritoneal macrophages, it also induced proinflammatory cytokine production after cross-linking with mAbs.[74]

## Ligand recognition of the CD300 molecules

To search for cells expressing the ligands for the CD300 molecules, several groups have used CD300-Fc chimeric proteins as a tool.[42,51,58,59,75-77] An interesting finding was that both CD300a-Fc and CD300f-Fc bind to dead cells from several species in a $Ca^{++}$–dependent manner,[42,59,76] suggesting that these 2 receptors must bind evolutionary conserved ligands.

Apoptotic/dead cells are characterized by changes in the plasma membrane, including the loss of phospholipid asymmetry.[78] From the early stages of apoptosis, cells expose PS and PE in the outer leaflet of the plasma membrane,[78-82] acting as "eat-me" signals and leading to their engulfment by phagocytes.[83,84] The binding of CD300a-Fc to dead cells was blocked by milk-fat globule EGF-factor VIII, a ligand for PS, and by duramycin, a ligand for PE.[42,76] Subsequent experiments using a variety of techniques showed the direct binding of CD300a-Fc to purified PS or PS-containing liposomes.[42,76] In addition, human CD300a-Fc was also shown to bind purified PE and PE-containing liposomes, exhibiting a stronger binding than that to PS.[76] The functional recognition of purified PE was shown in a system using reporter cells expressing a CD300a-CD3ζ chimeric receptor.[76] The binding of CD300a-Fc to PE immobilized on a membrane was not observed in another study, although it is not clear if the authors used human CD300a-Fc or mouse CD300a-Fc in their experiments.[42] This is an important issue because a different publication has reported that mouse CD300a-Fc does not bind to any lipid.[85] To identify the residues that are involved in human CD300a binding to PS and PE, a molecular model was generated based on the crystal structure of TIM-4 complexed with PS.[76,86] The crystal structure of human CD300a is known,[14] and the metal ion and a molecule of PS or PE were placed in positions corresponding to the PS bound to TIM-4 structure. The model showed that PE and PS interact with CD300a residues that form a cavity where the hydrophilic heads of the lipids can penetrate.[76]

Early studies showed that mouse CD300f-Fc bound strongly to $CD4^+$ T cells and weakly to $CD8^+$ T cells.[51] However, these results were not reproduced by others.[77] In another study, it was shown that mouse CD300f-Fc binds to apoptotic cells, suggesting that CD300f also recognizes phospholipids exposed on the outer leaflet of the plasma membrane of dead cells. Indeed, this binding was blocked when the PS ligand annexin V was present in the assay.[59] In addition, CD300f-Fc exhibited binding to a subset of activated T cells that was also blocked by annexin V, correlating with the exposure of PS on the surface of activated lymphocytes, including T and B cells.[59,87,88] The capability of CD300f-Fc binding to purified PS and PS-containing liposomes was demonstrated using enzyme-linked immunosorbent assay, ultracentrifugation, and surface plasmon resonance assays.[59] Another PS ligand (ie, milk-fat globule EGF-factor VIII) is able to block the binding of mouse CD300f-Fc to purified PS coated on a plate.[59] Recently, another group has published that mouse CD300f-Fc binds only to ceramide immobilized on a membrane, but surprisingly, it did not bind other lipids, including PS, PE, SPC, or phosphatidylcholine (PC).[58] However, the same authors found that the same recombinant protein binds ceramide, PC, SPC, high-density lipoprotein, and low-density lipoprotein coated on a plate, and that reporter cells expressing a mouse CD300f-CD3ζ chimeric receptor functionally recognized the same group of lipids, but not PS or PE.[58] It is important to note that Choi et al[59] did not test the binding of mouse CD300f-Fc to ceramide. Other studies have shown that human CD300f-Fc and rat CD300f-Fc are also able to bind cells from the central nervous system.[75] The identity of the CD300f ligand expressed in these cells remains unknown. Finally, in an enzyme-linked immunosorbent assay with purified immobilized lipids on a plate and mouse CD300-Fc recombinant proteins, a study found that mouse CD300 receptors have overlapping but distinctive patterns of lipid binding specificity.[85] In addition, in a reporter cell assay, CD300lb/LMIR-5/CLM-7/mIREM-3 was shown to functionally recognize lipids.[85] However, some of the results from this study are not in complete agreement with those obtained by others.[42,58,59] The reason for the discordant results between the different publications is currently unknown, and without a doubt, more studies are required to characterize the fine specificity of lipid binding by the CD300 molecules.

What are the consequences and the significance of binding lipids by the CD300 receptors? Given that both PS and PE are expressed on dead cells,[78-84] the role of CD300a and CD300f in the removal of apoptotic cells has been studied.[43,59,76] Expression of mouse CD300f enhanced phagocytosis of apoptotic cells by the fibroblast cell line L929.[59] Because CD300f has been shown to work mostly as an inhibitory receptor,[11,46] it is unclear how this receptor promoted phagocytosis. One possible explanation might be that the amount of SHP-1, a phosphatase mostly expressed in hematopoietic cells, is very low in L929 cells and not able to block the PI3K-mediated activation signal that is also generated after ligation of CD300f.[53,54] Undoubtedly, experiments in primary cells expressing CD300f are required to determine if this receptor promotes, or rather blocks, the phagocytosis of apoptotic cells in physiological conditions. On the other hand, human CD300a inhibits the uptake of apoptotic cells by monocyte-derived macrophages and by CD300a-expressing L929 cells.[76] Defective removal of dead cells has deleterious consequences for the host, leading to the development of autoimmunity.[83] The nature of the immune response to cell death depends on which, where, and how cells die, as well as what immune cells interact with them.[89] Variations in these parameters will determine whether cell death is immunogenic, tolerogenic, or silent.[89] It is possible that the CD300 family of receptors may play a central role in determining the outcome of the immune response when dead cells interact with different components of the immune system. Indeed, recently published data support this hypothesis.[43,67,68] For instance, CD300a inhibited the cytokine production by LPS-stimulated mast cells and macrophages in the presence of apoptotic cells.[43]

Extracellular ceramides are abundant in stomach, skin, and brain. In the skin, they are abundant in the epidermis, whereas disseminated or patchy expression is also found in the dermis, in the vicinity of mast cells.[58] In wild-type mice, the administration of an anticeramide Ab increased the FcεRI-mediated passive cutaneous anaphylaxis (PCA) reactions, whereas administration of ceramide liposomes diminished the PCA responses. The administration of both reagents had no effect in the $CD300f^{-/-}$ mice, indicating that the interaction between extracellular ceramides and CD300f negatively regulates FcεRI-mediated mast cell activation.[58]

TIM-1 and TIM-4 have been proposed as the endogenous ligands for CD300lb/LMIR-5/CLM-7/mIREM-3.[68] Interestingly,

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the binding of a CD300b-Fc protein to cells expressing TIM-1 with mutated Trp-Phe-Asn-Asp metal-ion PS-binding motif was completely abolished.[68] Although this finding may suggest that CD300lb/LMIR-5/CLM-7/mIREM-3 could interact with PS, it was shown that CD300b does not bind to phospholipids, does not promote phagocytosis of apoptotic cells, and does not affect phagocytosis mediated by TIM-1 or TIM-4.[68] These results are in contradiction with those showing that CD300lb/LMIR-5/CLM-7/mIREM-3 binds a specific pattern of lipids, including PS, PE, and PC.[85] Different methods used to determine the binding of the receptor to lipids may explain these opposing results. Nevertheless, very recently it has been proposed that an interaction between mouse CD300lb-Fc and an unidentified ligand, other than TIM-1 and TIM-4, is involved in CD300lb-Fc–induced cytokine production by peritoneal macrophages.[67]

## Disease relevance of the CD300 molecules

The expression of CD300a on mast cells, eosinophils, and basophils, 3 important cell types involved in the initiation and regulation of allergic responses, led to the design of bispecific antibody fragments targeting CD300a along with other receptors with the goal of downregulating the function of these cells during disease conditions.[90] Linking CD300a with c-Kit by a bispecific antibody fragment completely abrogated mast cell degranulation induced by SCF in a murine model of cutaneous anaphylaxis.[44] Another bispecific antibody fragment linking CD300a to IgE bound to FcεRI (specific for mast cells and basophils) abolished the allergic and inflammatory responses in 2 different animal models (ie, IgE-dependent PCA and ovalbumin induced acute experimental asthma).[45] A third bispecific antibody fragment linking CD300a to CCR3 (specific for mast cells and eosinophils) was shown to reduce eosinophil signaling in vivo, to inhibit bronchoalveolar lavage fluid inflammation, eosinophil and mast cell mediator release, eosinophil-derived TGF-β1 in the bronchoalveolar lavage fluid and lung remodeling, and very importantly, it reversed lung inflammation in a model of chronic established asthma.[91] Also, in a model of allergic peritonitis, neutralization of CD300a resulted in a significant increase of inflammatory mediators and eosinophilic infiltration.[33] Finally, it has been shown that the basal expression of CD300a on basophils from patients allergic to birch pollen is significantly lower than in healthy control individuals.[36] Altogether, these results indicate that CD300a functions as an inhibitory receptor in vivo and that it is a potential target for treatment of allergic diseases. In addition to their role in allergic processes, mast cells are also known to play an important role in a cecal ligation and puncture peritonitis model in mice,[92] where a large number of cells undergo apoptosis in the peritoneal cavity.[93] After cecal ligation and puncture, CD300a-deficient peritoneal mast cells produced more chemoattractants, leading to increased neutrophil recruitment and better bacterial clearance. As a consequence, CD300a$^{-/-}$ mice showed prolonged survival.[43] These results indicate that CD300a regulates mast cell inflammatory responses to microbial infections.

The CD300 gene complex has been linked to PSOR2, a susceptibility locus for psoriasis.[94,95] A single nucleotide polymorphism that encodes for a nonsynonymous polymorphism (R94Q) within the Ig domain of CD300a has been associated with susceptibility to psoriasis,[94] although subsequent studies have disputed this linkage. Nevertheless, it is important to mention that the surface expression of CD300a on CD4$^{+}$ T cells is lower in psoriatic patients compared with healthy controls,[27] and that a CD300a-Fc recombinant protein with arginine at position 94 binds better to dead cells, and to PE and PS, than a protein with glutamine at position 94.[76] Other genetic association studies have shown that CD300a may be involved in the etiology of Alzheimer disease.[96]

In combination with 3 other genes, CD300a has been proposed as a blood-based biomarker that can differentiate ulcerative colitis from Crohn disease and noninflammatory diarrhea.[97] This may help the current serological testing for inflammatory bowel disease that is based on reactivity to bacterial antigens and not host gene expression.[98] In genome-wide expression studies of lymphoblasts compared with normal CD19$^{+}$CD10$^{+}$ B-cell progenitors, CD300a was identified, along with other markers, to be differentially expressed in acute lymphoblastic leukemia.[99] Sixteen differentially expressed markers, including CD300a, were validated for minimal residual disease detection by 4-color flow cytometry analysis including antibodies against CD19, CD10, and CD34 and one of the new markers.[99]

During HIV infection, the expression of CD300a on B cells is deregulated, suggesting the possibility that this receptor may contribute to the B-cell dysfunction observed in HIV-infected patients.[30] Also, in HIV-specific CD8$^{+}$ T cells, it was found that the expression of the basic leucine transcription factor, ATF-like significantly correlates with the expression of CD300a and other inhibitory receptors.[26] Basic leucine transcription factor, ATF-like is upregulated in PD-1$^{+}$ exhausted HIV-specific CD8$^{+}$ T cells and inhibits T-cell function, probably by a mechanism that involves, among others, increased expression of inhibitory receptors such as CD300a. Finally, it has been reported that the frequency of CD8$^{+}$ CD300a$^{+}$ T cells, which are mainly effector and effector/memory CD8$^{+}$ T cells, is increased in the blood of pregnant women with chronic chorioamnionitis.[31] However, the biological significance of this finding is unknown.

In a model of multiple sclerosis, CD11b$^{+}$CD11c$^{+}$CD300f$^{+}$ cells derived from circulating inflammatory monocytes are recruited to the areas of inflammation in the central nervous system. These cells express iNOS and TNF-α. CD300f$^{-/-}$ mice or animals treated with a CD300f-Fc fusion protein presented a more severe disease without an increase in the incidence. This was characterized by an augmented release of myeloid-specific inflammatory mediators and an exacerbated demyelination. Lack of CD300f does not affect T-cell priming, indicating that this receptor regulates disease severity at the effector phase by suppressing the release of reactive oxygen species and inflammatory cytokines from myeloid effector cells.[77] The neuroprotective role of CD300f has also been shown in a rat model of acute brain injury.[75] Injection of *N*-methyl-D-aspartate into the striatum and cortex of rats induced an acute excitotoxic injury that was significantly reduced if a modular recombinant gene therapy vector bound to plasmids encoding human or rat CD300f was introduced into the lesion site.[75] Although CD300f$^{-/-}$ mice show normal mast cell development, the absence of the receptor led to exacerbated mast cell–dependent allergic responses. These include enhanced FcεRI-mediated passive systemic anaphylaxis and PCA, and mast cell–dependent airway inflammation and atopic dermatitis. In this context, it is important to note that chronically inflamed skin and lungs tend to have higher levels of extracellular ceramides, a ligand for mouse CD300f.[58]

Downregulation of CD300f in DCs with short interfering RNA was shown to enhance the antitumor effect of immunization protocols in a mouse model of cancer.[51] Korver et al[48] have generated antihuman CD300f Abs that show complement-dependent cytotoxicity and antibody-dependent cell cytotoxicity activities in vitro against CD300f-expressing AML-derived cell lines and freshly isolated blasts from AML patients. In addition,

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these Abs exhibited an antigrowth effect in an established HL-60 xenograft tumor model, and they significantly reduced the engraftment of primary human AML cells.[48] These studies highlight the potential of targeting CD300f in tumor immunotherapy.

In a model of renal ischemia/reperfusion, TIM-1 expression in the kidney was induced, and CD300lb/LMIR-5/CLM-7/mIREM-3–expressing neutrophils were recruited causing renal tubular damage. Both the recruitment of neutrophils and the kidney damage were significantly reduced in CD300lb/LMIR-5/CLM-7/mIREM-3–deficient mice, indicating a role for this receptor in these processes.[68] In addition, it has been reported that a soluble form of CD300lb/LMIR-5/CLM-7/mIREM-3 has a role in LPS-induced sepsis.[67] Serum levels of this soluble receptor increased after LPS stimulation or peritonitis induction, and intraperitoneal administration of CD300lb-Fc, a surrogate of soluble CD300lb/LMIR-5/CLM-7/mIREM-3, induced proinflammatory cytokine production by peritoneal macrophages. CD300lb/LMIR-5/CLM-7/mIREM-3–deficient mice are more protected against LPS- or peritonitis-induced lethal inflammation than wild-type mice, and importantly, they showed an increased mortality if they were injected with CD300lb-Fc, confirming the role of the soluble form of this receptor in this disease.[67]

## Future considerations

The development of better reagents to unequivocally detect and quantify the expression of the different members of the CD300 family is of great need. Another important area of future research should be the identification of ligands for the still "orphan" receptors, especially human CD300 molecules. Little is known about the topology of PS and PE exposed on apoptotic cells and how they are engaged by specific receptors, including the CD300 molecules. Furthermore, there is no information on whether CD300 receptors recognize ligands as monomers, dimers, or higher-order oligomers. We are at the starting point of understanding the role of the CD300 family of receptors in disease settings, the possibility of using them as biomarkers, and their potential as therapeutic targets. In light of the preclinical data obtained from mouse models, we need to know more about the involvement of the CD300 family in human diseases such as cancer, sepsis, and autoimmune and allergic diseases. Through their binding to lipids, the CD300 family forms an arrayed receptor system that is able to recognize the viability and activation status of cells and, consequently, have a significant influence on the final outcome of the immune response.

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*Note added in proof.* Since the original submission of this review, Takahashi et al. have shown that human CD300c is expressed on the cell surface of monocytes and mast cells, is able to deliver an activating signal after cross-linking with specific mAbs and recognizes the aminophospholipid PE (Takahashi et al. Human CD300C delivers an Fc receptor-gamma-dependent activating signal in mast cells and monocytes and differs from CD300A on ligand recognition. *J Biol Chem*, in press, 2013).

## Authorship

Contribution: F.B. wrote the manuscript.

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