



## Molecules in focus

# CD48: A co-stimulatory receptor of immunity

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## ABSTRACT

The CD48 molecule is a glycosyl-phosphatidyl-inositol (GPI)-anchored cell-surface protein of the CD2 family of molecules. Originally described on virally-induced B cells, CD48 has been found on various hematopoietic cells, and its expression is regulated by viral and bacterial products and immune-associated proteins. CD48 binds CD2 and other molecules, yet its high-affinity ligand in both mouse and human systems is 2B4. Despite its lack of an intracellular domain, stimulation of CD48 induces rearrangement of signaling factors in lipid rafts, Lck-kinase activity, and tyrosine phosphorylation. As an adhesion and co-stimulatory molecule, CD48 induces numerous effects in B and T lymphocytes, natural killer cells, mast cells, and eosinophils. Some of these depend upon cell-cell interactions via 2B4-CD48 binding. The structural and phenotypic characteristics of CD48, and its role in physiological and pathophysiological processes, are reviewed herein. Possible CD48-based applications for immune-impaired and inflammatory disorders are discussed as well.

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## 1. Introduction

CD48, a CD2-like molecule, is a glycosyl-phosphatidyl-inositol (GPI)-anchored protein (Boles et al., 2001) also referred to as human Blast-1, or BCM1 in mice and OX45 in rats (Wong et al., 1990). CD48 was first discovered in 1982 as a viral-induced differentiation antigen on human transformed B lymphoblasts (Thorley-Lawson et al., 1982). Since then, the 40–45 kD protein has been found on the surface of several hematopoietic cells, yet exists also in soluble form (Boles et al., 2001). CD48 is a ligand for the immunoreceptors CD2 and CD244 (2B4). Its activation involves signaling molecules such as Src kinases and caveolin (Shin and Abraham, 2001). Over the years, CD48 has been defined as an adhesion protein and a co-stimulatory factor (Boles et al., 2001), and its role extends from innate responses to bacteria to allergic inflammation (Shin and Abraham, 2001; Minai-Fleminger and Levi-Schaffer, 2009).

## 2. Structure

An immunoglobulin (Ig)-like receptor, CD48 belongs to the distinct CD2 subfamily that includes also CD2, CD58, CD244 (2B4), Ly9 and SLAM (Boles et al., 2001). The human gene encoding

CD48 is located on chromosome 1 at 1q21–23 (see Genbank accession number AL121985), close to other CD2 receptors, and murine CD48 is similarly positioned on chromosome 1 (Kingsmore et al., 1995). While allelic polymorphisms for CD48 between different mice strains exist, no functional consequences of this variability are known (Gonzalez-Cabrero et al., 1999).

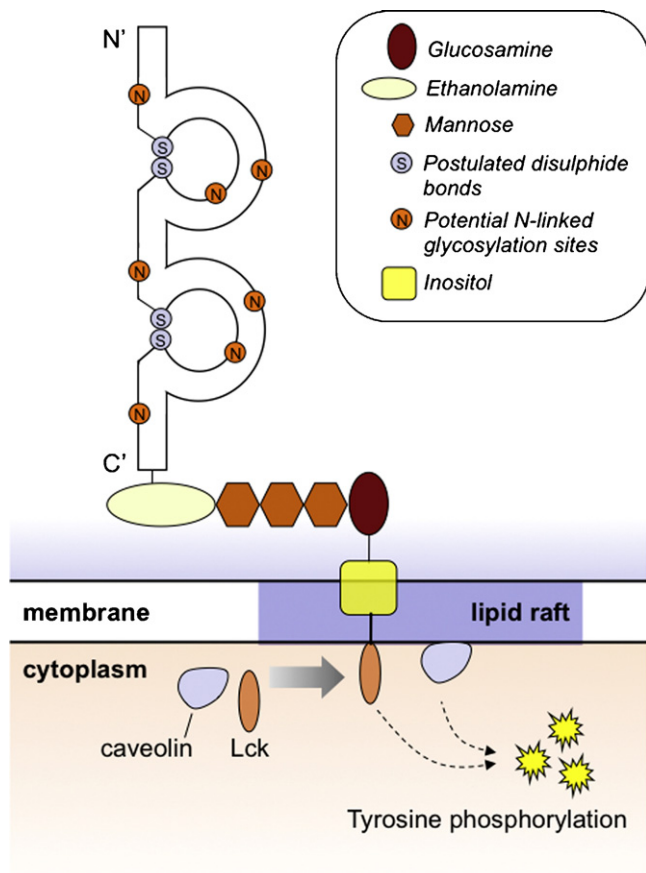
Like other CD2 molecules, the CD48 structure (Fig. 1) combines a distal V-like domain with a C2-like domain containing conserved cysteine residues that form disulfide bonds (Boles et al., 2001). A unique feature of CD48 within the CD2 group, shared only by CD58, is the lack of a transmembrane domain. Instead, CD48 is attached to the cell surface by a glycolipid, GPI, restricted to the outer leaflet of the membrane bilayer. The C-terminus of the polypeptide is covalently bound to ethanolamine, linked to an oligosaccharide containing mannose and glucosamine. The oligosaccharide binds the inositol head of GPI in the membrane. GPI molecules cluster in lipid rafts, thus CD48 is highly motile and aggregates in large microdomains involving signaling protein complexes (Shin and Abraham, 2001). Due to its GPI structure CD48 may be cleaved after activation, explaining the existence of soluble CD48 in circulation (Boles et al., 2001).

## 3. Expression, activation and turnover

CD48 exists on the surface of B and T lymphocytes, natural killer cells (NK), dendritic cells (DC), monocytes, neutrophils, mast cells (MC) and eosinophils (Eos) (Thorley-Lawson et al., 1982; Yokoyama et al., 1991; Katsuura et al., 1998; Boles et al., 2001; Shin and Abraham, 2001; Assarsson et al., 2005; Munitz et al.,

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**Fig. 1.** Structure and signaling cascade of CD48. The C-terminal amino acid of the polypeptide is attached to the cell membrane by ethanolamine, which is linked to an oligosaccharide that consists of mannose and glucosamine residues. The oligosaccharide is in turn bound to the inositol head group of phosphatidylinositol, which is embedded within the plasma membrane. Stimulated CD48 associates to the kinase Lck and leads to tyrosine phosphorylation, as well as redistribution and clustering of caveolae in the lipid rafts.

2006; Chlewicki et al., 2008). Endothelial cells (EC) are also a source of CD48 (Khan et al., 2007). On B cells, CD48 expression is augmented by the Epstein-Barr virus (EBV), through one of its proteins acting on an enhancer element in the CD48 promoter (Thorley-Lawson et al., 1982; Klamann and Thorley-Lawson, 1995). CD48 is upregulated also by interferons and virus-associated cytokines in several cells (Munitz et al., 2007a). In our studies, bacterial infection increased CD48 expression on MC (Rocha-de-Souza et al., 2008). Granulocyte-Colony Stimulating Factor and Interleukin (IL)-4 respectively induce CD48 on neutrophils and B cells (Yokoyama et al., 1991; Katsuura et al., 1998), while IL-3 increases the protein on Eos (Munitz et al., 2006).

Concomitant with the notion that CD2-family proteins interact among themselves, CD2 was the first identified human CD48 ligand (Boles et al., 2001). 2B4, a T and NK receptor, was subsequently defined as the high-affinity and more relevant ligand for CD48, with a 10-fold stronger interaction compared to the CD2-CD48 pair. The 2B4-CD48 couple appears highly conserved, as human and mouse 2B4 engage CD48 at similar affinities and docking topologies (Chlewicki et al., 2008). Notwithstanding, other ligands may exist: heparan sulfate on epithelial cells was found to engage CD48 (Boles et al., 2001). CD48 is also a MC receptor for *E. coli* FimH (Shin and Abraham, 2001).

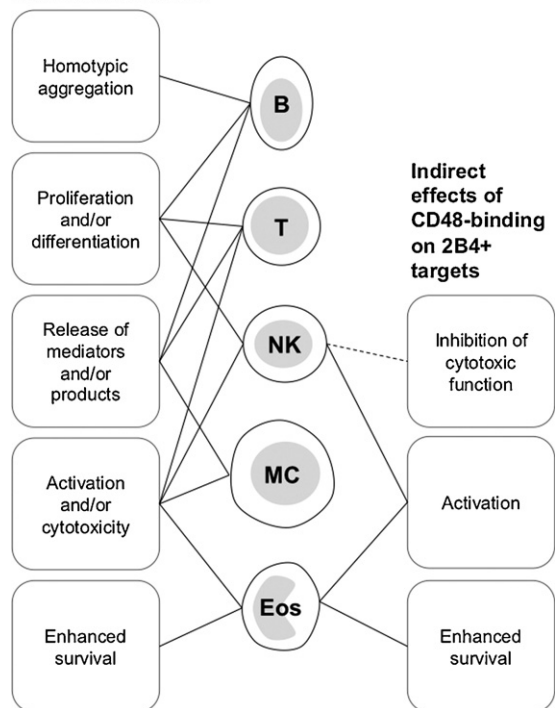
Despite the absence of a cytoplasmic domain, CD48 conveys a potent signaling cascade comparable to that of other immune-regulating molecules (Shin and Abraham, 2001). Several signaling

factors (e.g. Src tyrosine-kinases) are abundant in GPI-containing lipid rafts. CD48 co-precipitates with the Src kinase Lck, and CD48 cross-linking by antibodies (Abs) results in significant phosphorylation of tyrosine residues (Boles et al., 2001). Kinase activity is abolished under GPI-cleaving conditions, emphasizing the importance of the CD48 structure for its signaling ability. Since bacterial activation of CD48 involves clustering of caveolae in which it resides, phosphorylation and redistribution of caveolin and associated signaling factors is possible (Shin and Abraham, 2001). Interestingly, CD48 contributes to reorganization of molecules and signal transduction in T cell immunological synapses by binding to CD2 (Milstein et al., 2008).

#### 4. Biological function

Several CD48 functions have been discovered over the last decades (Fig. 2). Together with CD40, IL-4 and/or IL-10 stimuli, CD48 cross-linking by Abs causes B cells to aggregate, proliferate, differentiate and/or release Ig (Boles et al., 2001). In neutrophils, similar CD48 activation leads to increased cytoplasmic calcium levels (Horejsi et al., 1998). The co-stimulatory effect of the molecule was more extensively investigated in T lymphocytes: CD48 cross-linking increased intracellular calcium in human T cells (Stulnig et al., 1997) and proliferation of mouse CD3-stimulated CD8+ T cells (Kato et al., 1992). Concomitantly, CD48 blockade by soluble and immobilized Abs impaired T cell proliferation, IL-2 synthesis and receptor expression, and T cell receptor and cytoskeleton rearrangement (Kato et al., 1992; Stulnig et al., 1997; Marmor et al., 1999). Cytotoxic T lymphocyte activity is reduced upon CD48 Ab administration *in vitro* and *in vivo* (Chavin et al., 1994). Indeed, T cells from CD48-deficient mice have lower proliferative capacity and secrete less IL-2 upon activation (Gonzalez-Cabrero et al., 1999;

#### Direct effects of CD48 stimulation on cells



**Fig. 2.** Biological functions of CD48 in human and mouse. The major effects of CD48 stimulation on cells that express the molecule are indicated on the left. The influence of CD48 binding of 2B4, its ligand, on some of these cells, is shown on the right. Dashed connections indicate functions that appear only in the murine system. Abbreviations: NK, natural killer cells; MC, mast cells; Eos, eosinophils.

Lee et al., 2006). NK display similar attenuated functionality upon CD48 neutralization (Chavin et al., 1994; Assarsson et al., 2005), and increased cytotoxicity following CD48 Ab activation (Messmer et al., 2006).

The functions of CD48 were also studied in cell–cell interaction assays involving 2B4-positive cells. 2B4-binding of CD48 on adjacent T cells resulted in expansion and enhanced cytotoxicity of CD8+ lymphocytes (Assarsson et al., 2005). CD48 on NK is also activated by 2B4-expressing target cells (Messmer et al., 2006). Yet the CD48–2B4 interaction is bi-directional; In fact, most studies focused on CD48 as a ligand of NK 2B4. CD48 ignites human NK cytotoxicity via 2B4-interactions (Veillette, 2006), and is responsible for 2B4 relocation within the immunological synapse, a critical prerequisite for its activating functions (Roda-Navarro et al., 2004; Veillette, 2006). Similar 2B4-mediated NK activation exists in murine systems (Vaidya and Mathew, 2006; Veillette, 2006; Chlewicki et al., 2008), though the opposite effect was also convincingly demonstrated: CD48-positive tumor cells inhibited the lytic potential of NK (Vaidya and Mathew, 2006). Lysis of mouse DC was also reduced following NK 2B4 binding by CD48 on DC (Chlewicki et al., 2008).

It has been argued that both inhibitory and activatory 2B4 effects are possible, and that in a given 2B4-positive cell, the mechanism depends on its expression level, degree of cross-linking, and signaling cascade (Chlewicki et al., 2008). Thus, CD48 may sway NK activity to either outcome. Another explanation for the contradiction between murine and human NK 2B4 is that its regulation by CD48 underwent evolutionary changes (Vaidya and Mathew, 2006): In line with the wide CD48 expression pattern, the 2B4–CD48 axis was originally an MHC-independent “self” recognition pathway, preventing NK killing. Accordingly, EBV-induced CD48 expression may have constituted an early viral mechanism, inhibiting NK. Due to the significant viral load, an activating NK phenotype was gradually selected for in humans, rendering EBV-infected B cells susceptible to NK killing. Indeed, most patients suffering of X-linked lymphoproliferative disease (in which 2B4 has an inhibitory role) demonstrate high EBV incidence and severity (Veillette, 2006). In view of this, HIV-induced CD48 expression (Ward et al., 2007) may be a counter attempt to avoid NK lysis, representing a new mechanism allowing sophisticated virus strains to persist.

In MC, CD48 was initially studied in the innate immunity context. The CD48–interaction with FimH induces MC degranulation, TNF $\alpha$  release and bacterial uptake (Shin and Abraham, 2001). We similarly showed that MC infection by *S. aureus*, and subsequent release of mediators, is facilitated by CD48 (Rocha-de-Souza et al., 2008). A role for the protein in binding and penetration of *E. coli* to brain microvascular EC was likewise described (Khan et al., 2007). CD48 may affect MC functions also in allergy; IgE-mediated MC secretion of IL-8 is increased upon CD48 stimulation (our unpublished data). CD48 also activates Eos to degranulate (Munitz et al., 2006). The stimulatory ability of CD48 in MC and Eos, both key allergic effector cells, is supported *in vivo*: CD48 neutralization reduced asthma hallmarks in mice (Munitz et al., 2007a). It is therefore not surprising that high CD48 levels present on Eos of asthmatics (Munitz et al., 2006). Elevated mRNA and protein levels also appear in murine asthma (Munitz et al., 2007a), in which CD48 has been defined a signature gene (Zimmermann et al., 2003).

How CD48 is activated in allergy remains an open question. Although any 2B4-expressing cell can potentially ligate MC CD48 during inflammation, we postulated that Eos are the most likely cell type for this task, considering their extensive tissue infiltration (Minai-Fleminger and Levi-Schaffer, 2009). In interacting MC–Eos, CD48 is indeed localized at contact sites (our unpublished data). Both Eos-enhanced IgE-activation of MC and MC-induced increase of Eos viability are effects attenuated by 2B4 Abs (our unpublished data). Thus, the 2B4–CD48 axis appears a mechanism by which MC and Eos bind and stimulate one another in late/chronic allergy.

## 5. Clinical applications and targeted therapeutics

CD48 may be an important biomarker of infectious and allergic disorders. Monocytes, neutrophils, and lymphocytes of patients with infections (Katsuura et al., 1998), and Eos of allergic donors (Munitz et al., 2006), display elevated CD48 levels. Soluble CD48 is detected in the plasma of patients suffering from arthritis infectious diseases, and lymphoid leukemia (Boles et al., 2001), supporting its diagnostic potential. The high CD48 level is probably a sign that immune protection against infection takes place. In tandem however, a pathophysiological role for CD48 in inflammatory and autoimmune disorders may have been assumed. Accordingly, CD48-antagonistic therapies (blocking Abs, recombinant decoy molecules, gene targeting, etc.) may be useful for treating allergy, inflammation, and autoimmune syndromes (Munitz et al., 2007a,b).

Still, CD48-based clinical modalities are yet to be developed. Although CD48 has been implicated in inventions targeting tumor antigens and in targeted drug delivery (Munitz et al., 2007b), these have not addressed its stimulatory functions. Dampening inflammation by CD48 neutralization appears, however, feasible; CD48 Abs attenuate murine asthma and colitis, and promote mouse tissue engraftment by suppressing immunity (Munitz et al., 2007a,b). CD48 therapeutics will require careful design to reduce inflammation without harming regulation of infectious diseases. Moreover, in view of the wide CD48 expression pattern, efforts must be devoted to developing selective and/or conditional treatments.

In summary, CD48 is an intriguing molecule with extensive capability to modulate immune reactions, and significant therapeutic potential as a result. Studies hitherto are only the tip of the iceberg, and future work is expected to uncover additional features of this receptor in immunity and beyond.

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