

# CD48 as a Novel Target in Asthma Therapy

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**Abstract:** CD48, a CD2-related surface molecule, emerges as a critical effector molecule in immune responses. CD48 has a striking array of biological functions, among them adhesion, pathogen recognition, cellular activation, and cytokine regulation. Still, it is surprising that this mysterious molecule has not yet met its proper use as a therapeutic target in exaggerated immune disorders like hematopoietic tumors, autoimmunity and allergic reactions. Recently, CD48 was investigated in our laboratory as an effector molecule in human eosinophil function and in asthma. In this review, we shall discuss the known aspects of CD48 biology and describe the recent advances regarding the role of CD48 in human disease. Moreover, we shall review inventions making use of CD48, and discuss recent patents and the potential of CD48 as a future target for the therapy of allergic and other diseases.

**Keywords:** CD48, asthma, immunotherapy, mast cells, eosinophils, cancer, marker, 2B4, CD2, phagocytosis.

## INTRODUCTION

### THE CD2 RECEPTOR SUBFAMILY

The T-cell antigen CD2 was first described when antibodies reactive with the molecule were shown to block erythrocyte rosetting, a process widely used to identify human T cells. Since its discovery CD2 has largely grown into a distinct subfamily belonging to the Ig-SF [1] which includes CD2, CD48, CD58, CD84, CD150, 2B4, SF2001, NTB-A and CS1 [2-4]. The typical characteristic of the CD2 subfamily is the extra-cellular domain area. An N-terminal variable (V) Ig domain lacking any disulfide bond is followed by an Ig constant 2 (C2) domain with two intradomain disulfide bonds [1]. All the CD2 subfamily members are glycoproteins with extracellular N-linked glycosylation sites but no recognized O-glycosylation sites [1,5]. These post-translational modifications might contribute to the homo- and heterotypic adhesion of CD2 family receptors. Indeed, some CD2 family members interact with themselves or with other members of the CD2 family. In fact CD58 is a high affinity ligand for CD2, whereas CD48 is a low affinity ligand for CD2 [1] but a high affinity ligand for 2B4 [6]. Other members of this receptor family do not have a defined ligand as yet.

The CD2 family receptors are expressed predominantly on hematopoietic cells especially T and B lymphocytes and NK cells and their expression and function has not been investigated on eosinophils as yet.

### CD48

CD48 is a GPI anchored protein [7] that exists in both a membrane-associated and a soluble form [8]. CD48 lacks a transmembrane domain, so how it transduces signals to the cell interior is an intriguing question [9]. A possible explanation is that glycosylphosphatidylinositol (GPI)-

anchored proteins are preferentially found in distinct microdomains on the cell membrane known as glycosphingolipid-cholesterol rafts [10]. These rafts are rich in glycosphingolipids, cholesterol as well as important signaling molecules such as Src family protein tyrosine kinases and G-proteins. Therefore, the close proximity of these signaling molecules may explain the capability of signal transduction [11,12].

Many observations indicated the direct role of CD48 in transducing stimulatory or co-stimulatory signals. Cross-linking of CD48 on the surface of rodent T lymphocytes induced mobilization of the intracellular calcium inositol triphosphate concentration [13]. T cell activation via CD48 combined with CD3 induced enhanced IL-2 release, T cell receptor signaling and cytoskeletal reorganization [14]. Furthermore, cross-linking of CD48 on the surface of rat or murine B cells induced activation [15]. In humans, cross-linking of CD48 on B cells significantly increased CD40-mediated activation. CD48 in combination with IL-4 and/or IL-10 was able to induce B cell aggregation, proliferation and IgG secretion [16]. In addition, CD48 can deliver an independent signal for B cell differentiation [17]. CD48 also mediates TNF production by dendritic cells [18]. All these clearly show the role of CD48 as a trigger or co-stimulator of immune functions.

Interestingly, CD48 has an inducible expression pattern; for example, it is up regulated after Epstein - Barr virus (EBV) infections in B cells [19]. Other stimuli such as phorbol esters or IL-4 are also involved in the increase of CD48 expression on B cells [20].

As mentioned, in the human system, CD48 is the low affinity ligand for CD2, and thus might modulate T-cell activation and function. This is indicated by CD48 deficient mice, which show significant defects in CD4<sup>+</sup> T cells.

Investigations on the role of CD48 on mast cells were primarily focused on the role of CD48 in innate immunity. CD48 on mast cells is a receptor for fimbrial adhesion

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molecules [21]. However to the best of our knowledge, so far the function of this cell surface receptor has not been studied in eosinophils or in asthma settings. As such, we hypothesized that CD48 can deliver independent or co-stimulatory signals for eosinophil activation and may play a fundamental role in eosinophil related diseases such as asthma.

### CD48 AS A TARGET IN IMMUNE DISORDERS

CD48 is still a mysterious molecule, although it likely plays critical roles in general immune functions as an adhesion molecule, as an activatory receptor for CD2-related molecules, and as a receptor for fimbrial adhesion molecules as said above. It is therefore tempting to assume a role for CD48 as a valuable target in immune disorders like hematopoietic tumors, autoimmunity, allergy and chronic inflammatory diseases.

However, a patent search we conducted revealed that CD48 is centrally indicated only in two out of six containing the term CD48. In these two, CD48 is used as a cell-discriminating moiety for the targeted delivery of effector molecules.

Hsing-Cheng *et al.* [22] use CD48 as a tumor-associated marker for their antineoplastic strategy. In their strategy the inventors employ bispecific antibody fragments targeting tumor antigens such as CD20, CD22 etc. In this case it should be considered that the fact that CD48 is widely expressed can dampen the antibody's selectivity. Nevertheless, the antibody's higher avidity to cells co-expressing CD48 and a second tumor marker is the compelling factor in this issue.

In another invention, Bejanin and Tanaka [23] employ CD48 as a mast cell-associated target for liposome-based drugs. As we have discussed, mast cells display extremely high expression of CD48, and therefore targeting these cells by CD48 would be very efficient. Still, it is important to consider the wide expression's impact on selectivity.

In conclusion, current inventions use CD48 merely as a marker for targeted treatment. Although efficient, this clearly indicates that CD48 has not yet been investigated seriously as an effector molecule. Importantly, as we discussed, CD48 exhibits plasticity in various conditions and stimuli. This plasticity should also be studied, especially when using this molecule as a target in disease.

### 2B4 AND CD48 ON HUMAN EOSINOPHILS

In our studies, we demonstrated that human eosinophils express a wide variety of CD2-subfamily receptors such as CD48, CD58, CD84, 2B4 and NTB-A. 2B4 and CD48 received the center of our attention mainly due to the fact that they are receptor-ligand pairs. Nevertheless, the identification of CD84, NTB-A, 2B4 (all sharing signal transduction properties) and SAP, in eosinophils opens an opportunity to elucidate eosinophil functions in X-linked lymphoproliferative (XLP) disorder and others. Moreover, 2B4 and NTB-A have been shown to both activate and inhibit NK cell functions [3,18,24,25]. Hence, it is tempting to speculate that the transition of eosinophils from damage to repair could be regulated at least in part by the ability of these receptors to activate (and cause tissue damage) or inhibit (and induce tissue repair) eosinophil functions.

Our data suggests that 2B4 is an activating receptor on human eosinophils. Cross-linking of 2B4 triggers eosinophil degranulation, cytokine release and induces cytotoxicity toward CD48<sup>+</sup> cells. In addition, since airway epithelial cells may express CD48 [26], it is possible that 2B4-CD48 interactions, between eosinophils and epithelial cells, trigger eosinophil activation and consequent epithelial damage. Collectively, our data suggests that 2B4 expression on eosinophils may be relevant to diseases beyond allergy and hypersensitivity.

Next, we characterized the expression and function of CD48 on eosinophils. Our studies revealed three major findings. First, cross-linking of CD48 was sufficient to induce eosinophil degranulation but not cytokine release. The issue of how eosinophils release specific stored proteins has been addressed only in the last decade [27]. Eosinophils can release stored proteins via three mechanisms:

1. Constitutive pathway- this pathway involves vesicular transport from the endoplasmic reticulum and Golgi to the cell membrane and mostly occurs for newly synthesized mediators.
2. Exocytotic degranulation- this pathway involves direct fusion of the eosinophil granule into the membrane and causes unspecific granule content release.
3. Piecemeal degranulation- this pathway appears to be the major secretory pathway of eosinophils and involves "budding" of new vesicles from eosinophil granule membranes. This pathway involves vesicular transport as well.

CD48 triggers piecemeal degranulation of eosinophils. Since CD48 is a GPI-linked protein it activates intracellular signaling cascades by reshuffling lipid rafts on the cell surface [10,11]. Our study is the first to demonstrate activation of eosinophils through a GPI-linked receptor.

Second is the finding that IL-3 specifically regulates CD48 expression on eosinophils. This phenomenon was found to be conserved to both murine and human cells, suggesting that IL-3 induces signal transduction independent of the IL-3/IL-5/GM-CSF common beta chain. Interestingly, cross-linking of CD48 on human eosinophils triggers EPO release but no cytokine release even in the presence of IL-3. We speculate that under certain circumstances IL-3 can potentiate the responses elicited by CD48. In support of this hypothesis, it is our observation that IL-3 enhances the ability of eosinophils to internalize *E-coli* via CD48 (Levi-Schaffer *et al.* unpublished observations). Furthermore, IL-3 has been shown to prime eosinophils and enhance cytotoxicity towards antibody-coated helminths [28]. Therefore, it is possible that CD48 together with IL-3 regulates the release of specific mediators that may be beneficial to the host in helminth and bacterial infections (i.e. innate mechanisms) but unfavorable in allergic settings.

Third, CD48 expression is increased in tissues and blood eosinophils obtained from asthmatic donors. This finding may be especially important in the context of defining disease-specific pathways that regulate eosinophil functions in asthma. Strengthening this observation is our findings that CD48 increased in the lungs of allergen challenged mice and

is a component of the asthma genome signature described recently by Zimmerman *et al.* [29].

Key regulators of the asthmatic response such as STAT6, IL-4 and IL-13 that have been shown to regulate several genes from the asthma genome list [30], do not regulate CD48 expression. In order to establish a role for CD48 and its ligands (CD2 and 2B4) in asthma we neutralized these epitopes prior to allergen challenge in a murine asthma model.

Strikingly, neutralization of CD48 abrogated eosinophilic inflammation, Th2 and proinflammatory cytokine expression, lung inflammation, mucus production and smooth muscle hyperplasia [31]. These effects of anti-CD48 treatment are only partially dependent on interactions with CD2 expressed on lymphocytes, as anti-CD2 treatment induced a relatively modest effect.

Corollary to this, IL-18 that has been previously described as a Th1 inducing cytokine interacts with CD48 in order to induce its signaling cascades. Yet, IL-18 was recently shown to promote Th2 cytokine production from T cells, basophils, mast cells NK cells. Furthermore, it induces IgE production and keratinocytes that produce pro-IL-18 manifest atopic dermatitis [32]. Thus, CD48 may inhibit allergic responses also via IL-18-CD48 interactions [33].

Although, this study reveals a prominent role for CD48 in allergen challenged mice, several limitations oblige our conclusions regarding the critical function of CD48 on eosinophils and in human disease to be cautious. In our experimental design anti-CD48 was administered systemically. Thus, it may mask the activities of CD48 on various cells such as mast cells, basophils, B cells, T cells and eosinophils. Furthermore, at the time of antibody administration, eosinophilia is not present yet since eosinophils are recruited approximately 6 hrs after the second allergen challenge [34]. In addition, the relevance of this study to human asthma is yet to be confirmed since in humans CD58 is the ligand of CD2 and not CD48 [1]. Last, our *in-vivo* data suggests that 2B4 does not participate in murine asthmatic response. However, due to its limited expression on murine cells and its boundless expression on human leukocytes it may play a significant role in human asthma.

Nevertheless, our observations that CD48 is upregulated on human eosinophils from asthmatic donors (tissue and peripheral blood) together with its abundant expression on lung eosinophils *in vivo* and the relatively low effect of anti-CD2 treatment reinforce our hypothesis that CD48 is critically involved in human asthma pathogenesis and is therefore a potential target for asthma therapy.

#### CURRENT & FUTURE DEVELOPMENTS

In exaggerated immune disorders such as hematopoietic tumors, autoimmunity and allergic reactions, a critical role is played by molecules normally mediating desired effector functions. Large groups of these molecules have been characterized and include adhesion molecules, activation receptors etc.

Among these CD48 has not received appropriate attention in spite of emerging evidence that indicate this molecule as a critical effector molecule in immunity.

The multiple functions that CD48 can play, in both innate and adaptive immunity, suggests an ancient origin. Indeed as we saw, CD48 is upregulated in a striking array of diseases and abnormal conditions, and it is tempting to hypothesize that CD48 functioned as a probe for the organism's state of health/disease. This is not the entire case, as the upregulation of CD48 could serve both as a signal to other cells that a breach of immunity is occurring, and as an effector molecule itself - when upregulated it facilitates the immune response. The possibility that CD48 mediates a transition between innate and adaptive mechanisms is captivating.

We speculate that CD48 neutralization will become a critical mode of treatment in a vast array of human diseases. Many approaches may be considered: neutralizing antibodies, silencing RNA, or recombinant decoy molecules. However, due to lack of information regarding the biology of CD48, it is still early to make educated guesses regarding the therapeutic cost/effect of this strategy.

We conclude that although much remains to be elucidated, CD48 emerges as a critical molecule in human immunity, and will most likely be of use in the treatment of many diseases.

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