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Dendritic Cell CD83: A therapeutic target or innocent bystander?

Charlene M. Prazma and Thomas F. Tedder²

Department of Immunology, Duke University Medical Center, Durham, NC

Abstract

CD83 represents an intriguing target for immunotherapy due to its preferential expression on mature DCs, the most efficient of antigen presenting cells. Based on its restricted expression pattern, structure, and the paucity of CD4⁺ T cells in CD83-deficient mice, multiple immunologically important functions for CD83 during immune responses have been proposed. Indeed, several studies have reported that CD83 blockade using soluble receptor constructs inhibits T cell responses *in vitro* and *in vivo*, can affect autoimmune disease development and progression, and can inhibit transplant rejection. However, others have not been able to reproduce some of these findings, and antigen presenting cells deficient in CD83 expression or expressing a mutated form of CD83 induce normal T cell responses *in vitro*. This review examines the controversy surrounding CD83 function, alleged CD83 ligands, the potential therapeutic utility of recombinant proteins targeting CD83 function, and the importance of soluble serum CD83. While the validity of multiple previous studies needs to be confirmed, CD83 remains a fascinating cell surface molecule with a unique pattern of expression that has multiple confirmed functions in regulating immune system development and function.

Keywords

CD83; CD83 ligand; dendritic cell

1. Introduction

Dendritic cells (DC) represent an exceptionally promising target for immunotherapy due to their central role in immunoregulation. However, only a limited number of surface molecules are preferentially expressed by DCs, with high-density CD83 expression serving as a marker for human DC maturation [1]. Recent studies have therefore focused on CD83 as a potential target for therapeutic intervention, with a flurry of exciting, yet often contradictory papers providing new insight into the potential of this target for a variety of applications [2]. In this issue, Pashine *et al* demonstrate that the picture may be more complex [3], with conclusions that differ from those of previous publications [4–11]. Unfortunately, regulating immune responses and autoimmune disease is rarely simple, and offers both therapeutic opportunities and traps that must be carefully explored before pursuit. The accompanying commentaries by

²Address correspondence to: Thomas F. Tedder, Box 3010, Department of Immunology, Room 353 Jones Building, Research Drive, Duke University Medical Center, Durham, NC 27710, phone (919) 684-3578, Fax (919) 684-8982, E-mail, E-mail: thomas.tedder@duke.edu.

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authors of recent papers investigating the therapeutic potential of soluble CD83 proteins further illustrate the complexities of this evolving debate. Thus, the focus of this review is to put this debate into context and to point-out where additional studies and new knowledge are needed so that CD83 can either move forward as a therapeutic target or remain an innocent but promising bystander.

2. CD83 Expression

CD83 is a cell surface marker predominantly expressed on mature human and mouse DCs [1,12–14]. Among the DC subsets identified in humans, CD83 is expressed by circulating DCs [12] and tissue DCs; including interdigitating reticulum cells within the spleen, Langerhan's cells within the epidermis, and DCs within the thymic medulla [1]. Freshly isolated blood DCs begin to express high levels of surface CD83 within 6 h of *in vitro* culture [12]. Monocyte-derived DC's also express CD83 following culture with GM-CSF and IL-4, but require activation with TNF- α to fully upregulate high level cell surface CD83 expression [15]. Additionally, CD83 expression is induced as monocytes emigrate into human leprosy lesions and differentiate into DCs [16]. In general, DC acquisition of high-level cell surface CD83 expression correlates with upregulation of HLA class II antigen expression, and serves as a selective marker for DC activation/maturation [12].

CD83 expression is detectable on human lymphocytes activated *in vitro* and is present at low levels on many human B and T cell lines [1,17–19]. More specifically, CD83 is not detectable at significant levels on circulating human lymphocytes or NK cells, but CD83 is detectable on blasting lymphocytes following mitogen stimulation. *In situ*, CD83 expression is only detectable on some germinal center lymphocytes with immunohistochemistry staining, although CD83 transcripts are detectable in activated human B cells and at lower levels in human brain and lung tissue [1,17]. The malignant cells of Hodgkin's disease and Epstein-Barr virus transformed lymphoblastoid cell lines also express CD83 [20,21]. Thus, low level CD83 expression by human lymphocytes can be induced upon cellular activation.

In mice, mature DC populations express cell surface CD83. Specifically, purified thymic DCs and bone marrow-derived DCs express CD83 transcripts [22,23], and lipopolysaccharide exposure induces CD83 expression by bone marrow-derived DCs [24]. Among DC subsets, CD83 is expressed at low levels by a small population of freshly isolated splenic and thymic CD11c⁺CD8 α ⁺ and CD11c⁺CD8 α ^{neg} conventional DCs but is not expressed by freshly isolated plasmacytoid DCs. Splenic and thymic conventional DCs upregulate cell surface CD83 within hours of maturation with lipopolysaccharide and splenic plasmacytoid DCs upregulate CD83 expression following overnight maturation with CpG and recombinant IL-3 [14]. Thus, CD83 expression by most DC subsets is common between humans and mice.

Activated mouse lymphocytes also express CD83, but at much higher levels than human lymphocytes [14,25–27]. Cell surface CD83 is rapidly upregulated and highly expressed by activated CD4⁺ T cells, CD8⁺ T cells, and B cells. Both CD4⁺ and CD8⁺ T cells rapidly upregulate cell surface CD83 following stimulation through CD3 and CD28 [14]. Cell surface IgM, CD40, or toll-like receptor-4 signaling induces rapid CD83 expression on B cells, which correlates with increased MHC class II and induced CD86 expression [14]. Lymphocyte activation *in vivo* induces CD83 expression on a population of B cells prior to CD69 expression, demonstrating that CD83 is an early marker for activated B cells [14,27].

The generation and analysis of CD83-deficient (CD83^{-/-}) mice were the first studies to demonstrate that CD83 is functionally important *in vivo* [22,23]. These studies revealed that CD83 is expressed by thymic epithelial cells (TECs), and regulates CD4⁺ thymocyte and T cell development. In fact, purified TECs (CD45^{neg}CD11c^{neg}CD205⁺) and purified thymic DCs (CD11c⁺CD205^{neg}) express CD83 transcripts at similar levels. Intrathymic injection of wild

type TECs into CD83^{-/-} mice confirms that TEC expression of CD83 regulates CD4⁺ T cell development [22]. The defect in CD4⁺ T cell development in CD83^{-/-} mice is also rescued by the transgenic overexpression of CD83 [23]. By contrast, a lack of CD83 expression by developing thymocytes in CD83^{-/-} mice does not significantly affect intrinsic T cell development, as revealed in bone marrow chimera studies [22,23]. However, mature CD83^{-/-} lymphocytes exhibit a survival defect in comparison with wild type lymphocytes, demonstrating an important function for CD83 expression in the periphery [14]. Thus, CD83 expression is essential for normal immune system development and maintenance.

3. CD83 Structure

CD83 cloned from a human cDNA library was originally identified as a new member of the immunoglobulin (Ig) superfamily, with one extracellular V-type Ig-like domain that is encoded by two exons [1,17]. Mature human CD83 (hCD83) is composed of 205 amino acids, with a predicted molecular weight of 23 kD [1,17]. However, hCD83 has a molecular weight of ~45 kD due to extracellular domain N-linked glycosylation [1]. The highly glycosylated status of CD83 was confirmed through de-glycosylation studies [28–31]. Further studies demonstrate that CD83 immunoprecipitated from a Hodgkin's disease-derived cell line (KM-H2) has a molecular weight of ~53kD, while a soluble CD83 protein of ~50 kD is detected in the supernatant fluid of cultured KM-H2 cells [32]. A molecular weight comparison between the non-reduced and reduced forms of immunoprecipitated CD83 demonstrates that membrane hCD83 is expressed as a noncovalently associated single chain molecule [1].

Mouse CD83 (mCD83) has 63% amino acid sequence homology with hCD83, with the greatest homologies between their transmembrane and cytoplasmic domains [28,33]. The extracellular Ig-domain of mCD83 is composed of 10 fewer amino acids than the hCD83 Ig-domain, resulting in a mature protein of 175 amino acids that still contains a V-type Ig-like domain [33]. While not endogenously phosphorylated [1], mCD83 and hCD83 cytoplasmic domains are rich in serine and threonine residues. Mature hCD83 and mCD83 also possess five cysteine residues within analogous positions in their extracellular domains, including a conserved cysteine residue near the transmembrane region [1,33]. The function of membrane-proximal cysteine residue is unknown although recombinant hCD83 extracellular domain protein (consisting of amino acids 20-145) can form dimers through the most membrane proximal cysteine residue [34]. CD83 has also been highly conserved throughout evolution with homologs in elasmobranch and teleost fish [35]. Thus, the cellular expression profiles and similar structural characteristics shared between CD83 and B7/CD28 family members lead to the proposal that CD83 facilitates intercellular communication between antigen presenting cells and T cells during immune responses [1].

4. CD83 ligands?

Details regarding the existence, expression, and characterization of hCD83 and mCD83 ligands remain ambiguous. Several laboratories using soluble recombinant fusion proteins composed of the extracellular domains of hCD83 or mCD83 report the expression of potential CD83 ligands on diverse cell populations [5–7,25,36].

The first description of a potential CD83 ligand used a recombinant fusion protein composed of the extracellular domain of mCD83 fused with the Fc portion of human IgG1 (mCD83-IgG1) in immunofluorescence assays [25]. Dimeric mCD83-IgG1 generated using a mammalian culture system was reported to bind a subpopulation of murine B220⁺ spleen cells found in BALB/c and XID mice, but not CD4⁺ cells. Cells that bound FITC-labeled mCD83-IgG1 were not present in B cell-deficient mice, which served as the negative control for these experiments. These alluring results suggest the presence of a CD83 binding partner on some mouse B cells.

Lechmann *et al* have identified a potential CD83 ligand on human immature and mature DCs generated *in vitro* [5,34]. For these studies, the extracellular domain of hCD83 (hCD83ext; amino acids 23–128) fused with glutathione S-transferase (GST) was expressed using a bacterial culture system. Soluble hCD83ext was generated by thrombin cleavage and GST removal. Presumably, hCD83ext is a monomer, since an alternate version of hCD83ext consisting of amino acids 20–145 forms a dimer [31,34]. Human CD83ext₂₀₋₁₄₅ retains reactivity with a rat anti-hCD83 monoclonal antibody (mAb; CD83-1G11) in Western blot assays, and is able to block CD83 expression during DC maturation *in vitro*. However, it remains unknown whether hCD83ext₂₃₋₁₂₈, hCD83ext₂₀₋₁₄₅, and membrane CD83 share similar conformations. These authors also fused the extracellular domain of hCD83 to the Fc portion of human IgG1 (hCD83-IgG1), with expression in a mammalian culture system. Although the purity and specificity of hCD83-IgG1 was not reported, it was shown to mediate dose-dependent DC binding when added to anti-human Fc-specific antibody coated-plates, while GST added to the plates provided a measure for background DC binding. To verify the specificity of DC binding to hCD83-IgG1-coated plates, hCD83ext₂₃₋₁₂₈ was added to the assays, which reduced DC binding to background levels. Although the statistical significance of this data was not demonstrated, these results suggest a CD83 ligand expressed by mature and immature DCs. While not shown, the authors report no binding of native CD4⁺ or CD8⁺ T cells to CD83-IgG1 using this assay system. Since prokaryotic expressed hCD83ext₂₃₋₁₂₈ blocked DC binding to hCD83-IgG1, these studies suggested that CD83 glycosylation is not necessary for its ligand binding function *in vitro*.

Scholler *et al* have also used a dimeric recombinant hCD83 fusion protein expressed in a mammalian culture system (hCD83-IgG1) to assess ligand expression [7]. They detected low level binding of hCD83-IgG1 by immunofluorescence staining on human monocytes and CD8⁺ T cells, and on the HPB-ALL and Jurkat T cell lines. B cells and monocytic cell lines did not bind hCD83-IgG1, nor did CD56⁺ natural killer cells or granulocytes. However, hCD83 cDNA-transfected colon carcinoma cells were shown to bind to HPB-ALL cells at higher levels than parental carcinoma cells, with intercellular binding blocked using the anti-hCD83 (HB15a) mAb. Also in these studies, CD83-IgG1 immunoprecipitated a 72,000 M_r glycoprotein from HPB-ALL cells, although binding was eliminated by neuraminidase treatment of the target cells. This suggested that CD83 ligand binding was sialic acid dependent, prompting the authors to conclude that CD83 is a sialic acid-binding lectin and member of the sialoadhesin/Siglec family. However, CD83 shares none of the canonical features that define this sialic acid-binding subfamily of mammalian lectins and should not be considered a member of this family.

Most recently, Hirano *et al* generated a recombinant hCD83-IgG1 fusion protein and a hCD83-IgG1 fusion protein containing the IgA tailpiece (hCD83dod) [36]. These dimeric and dodecameric fusion proteins were expressed in a mammalian culture system. Incubation of CD83 cDNA-transfected cells with hCD83dod blocked CD83 mAb (HB15e) binding in immunofluorescence assays, with the same results for the Jurkat and HPB-ALL cell lines. Faint hCD83dod binding was also detected on primary human CD4⁺ and CD8⁺ T cells stimulated using allogenic DCs, or CD3 mAb when combined with CD28 ligation, but not on resting T cells. Furthermore, a direct comparison in cell surface binding of the dimeric and dodecameric versions of hCD83 recombinant fusion proteins led the authors to conclude that the interaction between CD83 and its ligand is not avid enough to be detected by dimeric or monomeric forms of soluble CD83.

Despite the demonstration that CD83-IgG1 fusion proteins and hCD83ext can block ligand binding and function during *in vitro* assays, anti-hCD83 mAbs do not have known ligand-blocking or function-blocking activities [12,31]. The only exception was for the HB15a hCD83 mAb in one study, which inhibited the binding of CD83-transfected cells to HPB-ALL cells

[7]. Nonetheless, given the variety of CD83 mAbs that have been tested, the finding that all tested CD83 mAbs cross block each other's binding, and the small size of the CD83 extracellular domain, we would have expected that an intact CD83 mAb would mimic the blocking functions attributed to CD83ext or a CD83-IgG1 fusion protein. These findings in combination with the functional studies outlined below suggest the possibility that recombinant soluble CD83 proteins could bind ligands that are not normally engaged by membrane CD83.

5. Soluble CD83 in vivo

The severe deficiency in CD4⁺ thymocytes and T cell numbers, and the truncated survival of mature lymphocytes in CD83^{-/-} mice highlight the promise of this molecule as a therapeutic target [14,22,23]. However, dissecting CD83 function using recombinant CD83 fusion proteins *in vitro* [4,5,25,34,37] and *in vivo* [6,11] has generated a wealth of information that is sometimes difficult to reconcile between studies and with results from CD83^{-/-} mice. DCs from CD83^{-/-} mice function normally during mixed leukocyte reactions (MLR) [22,23] and during *in vitro* antigen presenting cell assays [38]. In addition, CD83 mAbs do not block human T cell proliferation induced by DCs during MLR assays [12] and have no inhibitory activity [5]. However, hCD83ext or CD83-IgG1 fusion proteins inhibit MLR assays in some studies, raising the question of whether soluble versions of CD83 act in the same functional manner as membrane expressed CD83.

Dimeric mCD83-IgG1 generated by Cramer *et al* using a mammalian culture system inhibits lymphocyte proliferation [25]. Specifically, the proliferation and IL-2 secretion of freshly isolated spleen cells from ovalbumin₃₂₃₋₃₃₉-specific T cell antigen receptor (OT-II) transgenic mice is inhibited slightly by the addition of mCD83-IgG1 to cultures. The degree of inhibition varied according to mCD83-IgG1 concentrations added, although lower IL-2 production was only observed as lower amounts of mCD83-IgG1 were added, but the small degree of inhibition and an absence of statistical analysis make interpretation of this data difficult.

Lechmann *et al* have found that prokaryotic expressed hCD83ext₂₃₋₁₂₈ and hCD83ext₂₀₋₁₄₅ have functional effects *in vitro* [5,34]. hCD83ext₂₃₋₁₂₈ inhibits DC-dependent allogeneic MLR and DC-stimulated proliferation of an influenza virus peptide-specific cytotoxic T cell line *in vitro* in a concentration dependent manner. GST purified in the same way as hCD83 ext was used as a negative control, as was bovine serum albumin (BSA) or untreated cells. hCD83ext₂₀₋₁₄₅ also inhibits DC dependent allogeneic MLR assays, however with the effective dose (10 µg/ml) of hCD83ext₂₀₋₁₄₅ being twice that reported with hCD83ext₂₃₋₁₂₈ [34]. Eukaryotic expressed hCD83-IgG1 coupled to beads also inhibited MLR assays, with uncoupled beads alone serving as a negative control [5]. Statistical analysis of inhibition levels of hCD83ext were not presented for these studies. Prokaryotic expressed hCD83ext₂₀₋₁₄₅ with a mutation removing the membrane-proximal cysteine is also a monomer with an impressive immunosuppressive activity on allogeneic T-cell proliferation in MLR assays [34]. Furthermore, soluble CD83 binding to mature DCs also changes their cytoskeletal arrangement [37]. Soluble CD83-treated DCs round up and have only short, truncated, or no veils at all and are completely inhibited in their ability to form clusters with T cells. In these investigators hands, commercially available (Biocarta) mCD83-Ig fusion protein also inhibits mouse DC-driven allogeneic T cell proliferation [11].

Zinser *et al* have also assessed prokaryotic expressed hCD83ext function *in vivo*, using murine experimental autoimmune encephalomyelitis (EAE) as a model [11]. Three injections of hCD83ext substantially prevents the paralysis associated with EAE. In addition, hCD83ext treatment reduces leukocyte infiltration into the brain and spinal cord. Even when EAE is induced a second time, CD83-treated mice remain protected, indicating a long-lasting immunosuppressive effect. hCD83ext treatment even reduces paralyzes when treatment is

delayed until disease symptoms are fully established. BSA treatment was used as the negative control for these studies. This work is the most dramatic demonstration of an immunosuppressive role for soluble CD83, suggesting potent therapeutic potential in the regulation of autoimmunity.

Scholler *et al* also used hCD83-IgG1 expressed in a mammalian culture system to assess CD83 function [6]. When injected into mice transplanted with P815 mastocytoma cells, hCD83-IgG1 significantly enhanced the rate of tumor growth and inhibited the development of cytotoxic T cells. By contrast, mice immunized with a poorly immunogenic melanoma transfected to express CD83 prevented the outgrowth of wild-type K1735 cells. Coimmobilized hCD83-IgG1 and anti-CD3 mAb also enhanced human T cell proliferation and increased the proportion of CD8⁺ T cells within cultures, with media alone serving as the negative control. Enhanced proliferation in the presence of hCD83-IgG1 was not seen when purified T cells were used in this assay. A CD83-transfected human B cell line also stimulated T cell proliferation more effectively than untransfected cells in MLR assays, with hCD83-IgG1 suppressing these responses. The CD83-transfected human B cell line also increased the generation of cytolytic T cells during *in vitro* cultures. Although no means was used to demonstrate a direct effect of CD83 versus indirect effects from clone selection or potential secondary effects, these studies suggested that CD83 binding to ligand-bearing cells regulates the development of cellular immunity.

Luthje *et al* have generated transgenic mice expressing a soluble mCD83-IgG1 fusion protein under the control of the MHC class II promoter [39]. Soluble mCD83-IgG1 is detected in the serum and thymus lysates of transgenic mice by ELISA, with a consistent serum concentration in the range of 10–20 ng/ml. To assess CD4⁺ T cell function, mCD83-IgG1 mice were crossed with OT-II transgenic mice. CD4⁺ splenic and thymic T cells from mCD83-IgG1/OT-II double-transgenic mice have reduced proliferation, and IL-2 and INF- γ production in response to OVA₃₂₂₋₃₃₉ presentation by wild type antigen presenting cells. Additionally, mCD83-IgG1 transgenic mice infected with *Trypanosoma cruzi* display a higher parasitemia and a lower survival rate when compared with wild type control mice. CD8⁺ T cell function was normal in similar experiments. To assess whether altered T cell responses in mCD83-IgG1 transgenic mice were mediated by serum mCD83-IgG1 or defects in CD4⁺ T cell function, *T. cruzi* infected wild type mice were treated with recombinant dimeric mCD83-IgG1 isolated from COS cell cultures. However, mCD83-IgG1 treatment of wild type mice did not affect parasitemia or death rates when compared with mCD83-IgG1 transgenic mice, but 80% of the mice given recombinant mCD83-IgG1 succumbed to infection while 80% of wild type C57BL/6 mice survived. Although the statistical significance of this difference was not presented, the authors conclude that mCD83-IgG1 interfered with host immune responses. Thus, these studies suggest that CD4⁺ T cells generated in mCD83-IgG1 transgenic mice have an intrinsic defect in thymic selection due to blockade of epithelial CD83 interactions with thymocyte ligands, while the presence of mCD83-IgG1 in the periphery interferes with immune responses to infections. The only concern is that mCD83-IgG1 transgenic mice were derived from a single founder using DBA/2 embryonic stem cells and were backcrossed with C57BL/6 mice for 6 generations. Thus, it is not possible to rule out a genetic influence since all comparisons were made with wild type C57BL/6 mice and the effect linked DBA/2 genes might potentially have on the results was not assessed.

In recent mouse transplantation studies, Xu *et al* treated mice with a dimeric mCD83 fusion protein fused with the human Ig Fc tail which was expressed in a mammalian culture system [8]. CD83-Ig treatment delayed allograft rejection in fully MHC mismatched murine skin transplants with a corresponding decrease in serum INF- γ and IL-2 production by allograft infiltrating lymphocytes. These data and those from the aforementioned studies imply that blocking interactions between membrane CD83 and its ligand(s) or the direct binding of soluble

CD83 with its ligand(s) may modulate T cell activation during *in vitro* and *in vivo* assays. However, anti-hCD83 mAbs do not have ligand-blocking or function-blocking activities [5, 12]. Specifically, hCD83 mAbs do not inhibit DC-driven MLR assays or T cell proliferation *in vitro*. While it is possible that mAb engagement of CD83 might generate transmembrane signals that complicate blocking studies, studies have not been published showing that CD83 mAbs alter T cell function *in vivo*.

6. Serum CD83

Soluble CD83 is released into the supernatant fluid of cultured CD83⁺ cells, with small amounts of soluble hCD83 (mean values of 0.12–0.25 ng/ml) also found in the serum of healthy adults [32]. Serum CD83 is also detectable in some neonates [40]. Increased levels of soluble CD83 are found in synovial fluid from rheumatoid arthritis patients [41], and soluble CD83 was detected at levels >1 ng/ml levels in sera from some chronic lymphocytic leukemia or mantle cell lymphoma patients [42]. Serum ultracentrifugation does not diminish CD83 levels, arguing that serum CD83 does not reflect the presence of circulating exosomes or cell debris. That serum CD83 is similar in size to cell surface CD83 suggests that serum CD83 may be generated by proteolytic shedding of membrane-expressed CD83 ectodomain [32], although receptor cleavage remains to be demonstrated.

Only anecdotal evidence suggests that serum CD83 may have functional activity. Senechal *et al* demonstrate that infection of cultured mouse DCs with human cytomegalovirus reduces CD83 surface expression with a subsequent increase of CD83 in the culture supernatant fluid [43]. This supernatant fluid normally inhibits DC immunostimulation when added to *in vitro* assays. However, the inhibitory effect of the supernatant fluid derived from *in vitro* human cytomegalovirus infected mature DCs is reduced after immunodepletion using CD83 mAbs. Although these results suggest that soluble CD83 affects mature DCs, T cells, or both during allogenic MLR assays, the inhibitory activity of the culture supernatant fluid could also result from changes in additional factor(s) that were not measured.

Recent studies by Dudziak *et al* report the PCR amplification of three alternative hCD83 transcripts generated by the utilization of potential alternative splice sites in resting or activated blood mononuclear cells [4]. The predominant transcript species encodes full-length CD83. One potential truncated CD83 protein (termed CD83c) lacks the transmembrane domain, but exon III encoding the second major half of the Ig-like domain is also spliced out, preventing the production of a native protein with an extracellular Ig-like domain. The two other transcripts (CD83-b and CD83-a) also lack exon III or delete a small portion of exon III, but both cause frame shifts that generate proteins with different carboxyl-terminal ends. Since the truncated transcripts were commonly detected when CD83 mRNA levels were low in resting blood mononuclear cells, while full length CD83 transcripts were most commonly detected in activated mononuclear cells that would express CD83, these alternative transcripts could represent PCR artifacts incurred during the amplification of rare transcripts. At a minimum, it must be demonstrated that these alternative transcripts are present at physiologic levels. Regardless, when these three alternative transcripts were transfected into 293-T cells, only CD83-c generated a protein product [4]. This may explain why truncated CD83 proteins have not been identified in serum [32].

CD83-c transcripts are predicted to produce an ~8 kD truncated protein composed of only a small portion of the extracellular Ig-like domain fused with the cytoplasmic tail of hCD83, without N-linked glycosylation sites [4]. However, CD83-c purified from the culture supernatant fluid of cDNA-transfected 293-T cells is a 25–28 kD protein in Western blot assays using the HB15a mAb [4]. Remarkably, the CD83-c protein is reported to reduce cellular proliferation in allogeneic MLR assays. However, inhibitory results were demonstrated at only

one functional data point, the effects of control proteins or irrelevant cell surface targets were not studied, and the statistical significance of this data was not tested. Thus, studies are required to validate the existence and functional relevance of these putative alternative CD83 splice isoforms.

While it is intriguing to hypothesize that increased serum CD83 levels may dysregulate T cell function and contribute to disease pathogenesis, mechanisms describing how such low levels (<1 ng/ml) of soluble CD83 might regulate T cell function must be clarified. Recombinant CD83 protein concentrations necessary to inhibit T cell activation *in vitro* vary from 500 ng/ml [11] to 4,000 ng/ml [5] for CD83ext, to 20,000 ng/ml for CD83-IgG1 fusion proteins [25, 39]. Soluble forms of multiple cell surface molecules have been detected within the serum of normal individuals and in the serum of individuals with various disorders at much higher levels than CD83, yet the functional significance of these proteins remains to be elucidated in most cases [44]. For example, L-selectin ectodomain is found in human and mouse serum at 1–10 µg/ml levels [45,46]. Therefore, it is essential to determine whether serum CD83 levels are sufficient to compete with membrane CD83 for ligand binding. Based on the significantly higher concentrations of CD83-IgG1 and hCD83ext required to demonstrate functional effects *in vitro* and *in vivo*, this is highly relevant. Alternatively, it can be argued that localized soluble CD83 concentrations within tissues might be higher than those found within serum, although localized concentrations of membrane CD83 would also be higher within these microenvironments.

7. Membrane CD83 function

Membrane expressed CD83 may also function differently than soluble forms of CD83. For example, Hirano *et al* have shown that membrane bound CD83 delivers a significant signal specifically supporting the expansion of newly primed naïve CD8⁺ T cells [36]. This interaction enhances the *in vitro* generation of cytotoxic T cells specific for viral antigens and enables the long-term survival of antigen-specific T cell cultures by inducing proliferation and inhibiting apoptosis. This suggests that CD83 binding to its ligands delivers necessary signals for CD8⁺ T cell survival *in vitro*. Consistent with this, lymphocytes from CD83^{-/-} mice have shortened lifespans *in vivo* [14]. Thus, the use of CD83 expressing artificial antigen-presenting cells may be useful for cancer or infectious disease therapy.

Studies modulating membrane CD83 expression by DCs have also provided important insight into CD83 function. Specifically, allogenic T cell stimulation by human DCs is reduced when cell surface CD83 expression is down-regulation by RNA interference [19,47]. By contrast, the transient overexpression of CD83 by immature and mature human DCs enhances their T cell stimulatory capacity [19]. These results parallel studies where CD83 overexpression by a human B cell line and a mouse melanoma cell line enhances T cell proliferation and INF-γ production *in vitro* and *in vivo* [6,9]. However, other explanations are equally possible since cell surface CD83 expression can influence the surface molecule phenotype of cells. Although, CD83^{-/-} and wild type DCs are equivalent in their ability to induce proliferation in MLR assays, MHC class II and cell surface CD86 levels are significantly reduced on the surface of antigen presenting cells from CD83^{-/-} mice [22,38]. Reciprocally, the transgenic overexpression of CD83 leads to enhanced cell surface MHC class II and CD86 expression by mouse B cells [27]. Unfortunately, the molecular basis for CD83 expression regulating select cell surface molecules is not currently known. Regardless, altered immune responses elicited following modulation of membrane CD83 expression on DCs may result from induced alterations in other additional cell surface molecules.

8. The potential therapeutic role of soluble CD83

The study presented in this issue by Pashine *et al* further evaluates the *in vitro* and *in vivo* utility of soluble hCD83-IgG1 (amino acids 1-131). hCD83-IgG1 was generated in a mammalian cell system and exists as a dimer. Glycosylation of hCD83-IgG1 was confirmed by mass spectrometry and hCD83-IgG1 was shown to effectively block CD83 mAb binding to cell surface expressed CD83. Nonetheless, hCD83-IgG1 in these studies was unable to block allogenic human T cell proliferation in MLR assays and failed to reduce the severity of EAE in mice upon *in vivo* treatment. These results contrast markedly with published reports [5,11]. However, there are obvious differences in the proteins used in these respective studies. Primarily, protein production in mammalian versus bacterial culture systems results in differences in protein glycosylation and may inadvertently contribute different contaminants that could have unanticipated *in vivo* and *in vitro* effects. Second, the current studies assessed dimeric hCD83-IgG1 while the previous studies focused on monomeric hCD83ext. Whether these different proteins have different pharmacokinetics *in vivo* is also unknown, but it is hard to understand why hCD83ext would block *in vitro* MLR assays while hCD83-IgG1 would not. Since it is unknown whether hCD83 and mCD83 bind common ligands it also remains unknown whether one should expect a hCD83 reagent to block both human MLR assays and mouse EAE models. In fact, hCD83 and mCD83 are most dissimilar in their extracellular regions, suggesting a potential for different ligands. Zinser and *et al* attempt to address this concern by comparing the ability of hCD83ext and mCD83-IgG1 to inhibit allogeneic proliferation of murine T cells [11]. However, T cell proliferation induced by DCs preincubated with appropriate irrelevant control proteins was not tested in these studies making it difficult to assess the validity of the results.

8. Concluding remarks

The combined results from these studies indicate that a ligand for CD83 may exist. The variation in reported expression of a cellular ligand for CD83 likely reflects technical issues that are inherent with the use of recombinant fusion proteins as alluded to by Hirano *et al*. Additionally, the glycosylation status of soluble CD83 proteins is another factor that may affect the binding avidity of recombinant CD83 fusion proteins with potential ligands. Thus, it will be essential for these and other investigators to validate and unify the studies claiming to identify seemingly disparate CD83 ligands. In addition, activated human and mouse DCs express cell surface CD83 while mouse, but not human lymphocytes express high level CD83 acutely after activation [14,25]. This further complicates the interpretation of studies using soluble CD83 to block ligand binding in humans and mice and demonstrates the complex and possibly multifaceted roles for CD83 during immune responses.

The data from the aforementioned studies also imply that blocking interactions between membrane CD83 and its ligand(s) or the direct binding of soluble CD83 with its ligand(s) may down modulate T cell activation. Although the current and previously published studies do not conclusively demonstrate that CD83 is or is not a valid therapeutic target, this debate raises multiple important issues. Primarily, differences between the results of those investigating the therapeutic role of soluble CD83 emphasize the absolute need for investigators to use similar, if not identical reagents that are prepared and purified similarly. The identification and characterization of CD83 mAbs that block ligand binding is critical so that they can serve as negative controls. Likewise, identifying the ligand binding regions within the extracellular CD83 domain will enhance our understanding of CD83 function. These differing results further reveal the complexities inherent in identifying new therapeutic targets. It is common that early results from different studies do not agree, but normally the appropriate conclusions converge into a unified impression with only differences in details. Since the phenotypes of CD83^{-/-} and CD83 mutant mice generated by independent groups are similar [22,23], and since

therapeutic efficacy was demonstrated upon depletion of CD83 expressing antigen presenting cells with mAb therapy [48], CD83 undoubtedly remains an interesting target for the manipulation of immune responses.

In conclusion, the paper by Pashine *et al* illustrates the importance of publishing what could be considered by some to be negative results. While most authors, reviewers, editors, and journals display a negative attitude towards papers that can not reproduce earlier studies, these papers challenge the research community and purge the literature of findings that are not reproducible. From the data that are currently available, it is impossible to determine whether soluble CD83 has a therapeutic effect or whether the results from these groups are equally valid for unknown reasons. Thus, it is critical for those with interest to read the published papers, critically evaluate all data and conclusions, assess whether appropriate controls were utilized, consider the comments of all groups, and make their own determinations. Regardless of the conclusions, CD83 remains an intriguing target if appropriately harnessed.

Abbreviations

BSA	bovine serum albumin
CD83-IgG1	extracellular domain of CD83 fused to Fc portion of human IgG1
CD83ext	CD83 extracellular domain
DCs	dendritic cells
EAE	experimental autoimmune encephalomyelitis
GST	glutathione S-transferase
hCD83	human CD83
Ig	immunoglobulin
kD	kilodalton
mAb	monoclonal antibody
mCD83	mouse CD83
MHC	major histocompatibility complex
MLR	mixed lymphocyte reaction
OT-II	

transgenic mice expressing TCR specific for chicken ovalbumin 323-339

TEC

thymic epithelial cells

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