Crystal Structure of the Extracellular Domain of the Human Dendritic Cell Surface Marker CD83

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Abstract

CD83 is a type-I membrane protein and an efficient marker for identifying mature dendritic cells. Whereas membrane-bound, full-length CD83 co-stimulates the immune system, a soluble variant (sCD83), consisting of the extracellular domain only, displays strong immune-suppressive activities. Besides a prediction that sCD83 adopts a V-set Ig-like fold, however, little is known about the molecular architecture of CD83 and the mechanism by which CD83 exerts its function on dendritic cells and additional immune cells. Here, we report the crystal structure of human sCD83 up to a resolution of 1.7 Å solved in three different crystal forms. Interestingly, β-strands C′, C″, and D that are typical for V-set Ig-domains could not be traced in sCD83. Mass spectrometry analyses, limited proteolysis experiments, and bioinformatics studies show that the corresponding segment displays enhanced main-chain accessibility, extraordinary low sequence conservation, and a predicted high disorder propensity. Chimeric proteins with amino acid swaps in this segment show unaltered immune-suppressive activities in a TNF-α assay when compared to wild-type sCD83. This strongly indicates that this segment does not participate in the biological activity of CD83. The crystal structure of CD83 shows the recurrent formation of dimers and trimers in the various crystal forms and reveals strong structural similarities between sCD83 and B7 family members and CD48, a signaling lymphocyte activation molecule family member. This suggests that CD83 exerts its immunological activity by mixed homotypic and heterotypic interactions as typically observed for proteins present in the immunological synapse.

Introduction

CD83 is a type-I membrane protein expressed predominantly on human mature dendritic cells (DCs). CD83 is expressed, in addition, on subsets of activated T cells, B cells, myelocytes, neutrophils, granulocyte precursor cells, and thymus epithelial cells and on regulatory T cells [1–7]. As CD83 expression is strongly induced during DC maturation, CD83 is considered to be one of the best surface markers for mature DCs [8]. The highly glycosylated human CD83 protein is 205 residues long, has a molecular weight of approximately 45 kDa, and belongs to the Ig-superfamily [8].

While residues 1–19 encode for the signal peptide, residues 20–144 build up the extracellular domain that has been predicted to display a V-set Ig-domain fold [8,9]. The extracellular domain is followed by a membrane-spanning segment (residues 145–166) and a cytoplasmic tail domain (residues 167–205) [8].

Two naturally occurring CD83 isoforms have been described: a full-length and membrane-bound CD83 form and a soluble form (sCD83), consisting of the extracellular domain only [10]. Full-length, membrane-bound CD83 is vital for T cell development in the thymus since CD83 knockout animals have strongly reduced $CD4^+$ T cell numbers in the periphery [4,11]. Using *in vitro* small interfering RNA (siRNA) knockdown approaches in mature DCs, it has been demonstrated that these DCs show a significantly reduced stimulatory capacity *in vitro,* thereby suggesting co-stimulatory properties of CD83 when expressed on human mature DCs [12,13]. In addition, interference with the nuclear export of CD83 mRNA in DCs, by blocking the CRM1 pathway, led to a strongly reduced T cell stimulation [14]. Interestingly, overexpression of CD83 on naive effector T cells led to the induction of a regulatory phenotype [15]. Several viruses including human cytomegalovirus, varicella-zoster virus, and herpes simplex virus 1 reduce the cell surface presentation of CD83, thereby inducing specific immune evasion strategies, which lead to suppressed antiviral immune responses [16–18].

In contrast to membrane-bound and full-length CD83, the soluble form sCD83 was shown to possess potent immune-suppressive properties and is released from activated DCs and from B cells [19]. It has been demonstrated that *in vitro* sCD83 suppresses DC-mediated T cell stimulation, and *in vivo*, it inhibits disease-associated symptoms in animal models of experimental autoimmune encephalomyelitis, a model for the early inflammatory phase of human multiple sclerosis, in inflammatory bowel disease models and in SLE models [20–23]. Furthermore, sCD83 prevents graft rejection in several transplantation models including heart, kidney, cornea, and skin transplants [24–26]. These data highlight the potential immunostimulating activity of membrane-bound CD83 and the immune-suppressive activities of sCD83. The molecular mechanisms underlying these opposite activities, however, are currently not well understood.

The mechanism by which CD83 exerts its function shares possibly some parallels with that of other co-stimulatory and co-inhibitory molecules that participate in the formation of the immunological synapse such as the B7 proteins CD80 (B7-1) and CD86 (B7-2) [27,28]. The extracellular domains of these B7 proteins also contain V-set Ig-domains, and upon binding to two distinct receptors, namely CD28 and CTLA-4, they either co-stimulate or co-inhibit T cell function [28–30]. Interestingly, the receptors CD28 and CTLA-4 also consist of V-set Ig-domains, and complex formation with the B7 proteins relies on V-set Ig-domain interdomain contacts [31,32]. However, whereas in the case of the B7 proteins, the interactions partners are well characterized, no CD83-binding partner has yet been identified. The opposite activities of membrane-bound CD83 *versus* sCD83 would be fully in line with the former acting as an active receptor by itself. Signaling-inactive sCD83 could then antagonize signaling by sequestering the corresponding ligand, thereby preventing the ligand from activating membrane-bound CD83. Such opposite effects of soluble *versus* membrane-bound receptors are well documented for cytokine receptors [33]. However, with no CD83-binding partner identified so far, alternative models must also be considered. Thus, since CD83 is expressed on many different cell types, it is conceivable that CD83 promotes cell clustering through homotypic interactions [34]. In such a model, sCD83 could disrupt cell clustering by displacing individual membrane-bound CD83 molecules from homomeric complexes.

Here, we report the first crystal structure of human sCD83 up to a resolution of 1.7 Å solved in three different crystal forms. Structure determination, limited proteolysis experiments, and mass spectrometry analyses show that in the structure, β-strands C′, C″, and D that are typical for V-set Ig-domains are missing. Subsequent truncation and substitution experiments also show that this segment does not participate in the biological function of CD83. Structure comparisons reveal high structural similarity with CD48, programmed cell death ligand 2 (PD-L2), CD86, and CD80. Together with crystal packing analyses, these similarities suggest that CD83 exerts its function through mixed homotypic and heterotypic interactions as typically observed for proteins present in the immunological synapse. We expect that the establishment of the 3D structure of sCD83 and its ability and potential for complex formation provide the basis for more precisely understanding the molecular function of CD83 in the future.

Results

Crystal structure of variant sCD83

An *Escherichia coli* produced variant of human sCD83 (variant sCD83), in which three out of five cysteines were replaced with serines, crystallized in three different crystal forms. Whereas cubic crystals (space group $I4_132$, R_{work} = 23.7%, R_{free} = 26.4%) diffracted to only 3.2 Å, rhombohedral (R32, R_{work} = 17.2%, R_{free} = 21.6%) and trigonal crystals (P321, R_{work} = 18.8%, R_{free} = 21.6%) diffracted to 1.8 and 1.7 Å resolution, respectively (Table 1). The atomic models derived from the three crystal forms are highly similar (average pairwise main-chain RMSD =