Dealing with the family: CD147 interactions with cyclophilins

Vyacheslav Yurchenko,[1] Stephanie Constant[2] and Michael Bukrinsky[2]
[1]*Albert Einstein College of Medicine of Yeshiva University, Bronx, NY, USA and* [2]*George Washington University Medical Center, Washington, DC, USA*

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Correspondence: Dr M. Bukrinsky, GWU Medical Center, Ross Hall Rm. 734, 2300 Eye St. NW, Washington, DC 20037, USA.
Email: mtmmib@gwumc.edu
Senior author: Michael Bukrinsky

Summary

CD147 is a widely expressed plasma membrane protein that has been implicated in a variety of physiological and pathological activities. It is best known for its ability to function as *e*xtracellular *m*atrix *m*etallo*p*roteinase *in*ducer (hence the other name for this protein, EMMPRIN), but has also been shown to regulate lymphocyte responsiveness, monocarboxylate transporter expression and spermatogenesis. These functions reflect multiple interacting partners of CD147. Recently, interaction of CD147 with proteins of the cyclophilin family has been demonstrated and activity of CD147 as a signalling receptor to extarcellular cyclophilins A and B has been shown. Given that extracellular cyclophilins are potent chemotactic agents for various immune cells, further studies of the role of cyclophilin–CD147 interaction in inflammation followed. They demonstrated that agents targeting CD147 or cyclophilin had a significant anti-inflammatory effect in animal models of acute or chronic lung diseases and rheumatoid arthritis. Here, we review the current knowledge about interactions between CD147 and cyclophilins.

Keywords: EMMPRIN; receptor; intracellular trafficking; inflammation; adhesion

Introduction

CD147 is a ubiquitously expressed integral plasma membrane glycoprotein. It has been characterized under a variety of names in different species: rats (OX-47 antigen[1] and CE9[2]), mice (gp42[3] and basigin[4]), chickens (HT7[5] neurothelin and 5A11 antigen[6]). In humans, this protein was first described by Biswas and colleagues as a factor made by tumour cells that stimulates production of a collagenase (matrix metalloproteinase type 1, MMP-1) by fibroblasts.[7,8] This factor was identified and designated *tumour* cell-derived *c*ollagenase *s*timulatory *f*actor (TCSF).[9] Later, it was found that TCSF is expressed not only on tumour, but also on normal cells.[10,11] The same factor was described in other studies as hBasigin, M6, or Hab18G.[12,13] It is best known under the name *e*xtracellular *m*atrix *m*etallo*p*roteinase *in*ducer (EMMPRIN), as this property of the protein has been most extensively studied. For consistency, in this review we will refer to this protein by its recently adopted designation, CD147.[14]

CD147 is expressed at varying levels in many cell types, including haematopoietic, epithelial, endothelial cells and leukocytes.[1,11,15] The human CD147 is a 269 amino acid-long protein that belongs to the type I integral membrane protein family with a predicted molecular mass of ~28 000 MW.[10] The N-terminal extracellular part of CD147 consists of two C2 type immunoglobulin-like domains, which are heavily glycosylated. Recently, another form of CD147, containing an additional extracellular membrane-distal immunoglobulin-like domain, has been characterized.[16] This form was shown to be responsible for the majority of homophilic CD147 interactions. Endoglycosidase F treatment leads to a mobility shift from ~58 000 MW to ~28 000 MW, suggesting that the majority of CD147 glycosylation is N-linked.[12]

The 21-residue long transmembrane domain of CD147, represented by a hydrophobic stretch of amino acids interrupted by a charged residue, is almost identical among different species[17] suggesting its functional role beside anchoring CD147 in the membrane. Charged residues are not usually found in proteins spanning the membrane only once (as is the case for CD147), because a charged residue in the middle of the lipid bilayer is highly energetically unfavorable. This structural feature suggests that CD147 forms a complex with other membrane proteins thereby shielding the charge in an energetically stable state. Indeed, the transmembrane domain is

Table 1. CD147-interacting proteins

Protein	CD147 domain involved	Result of interaction	References
CD147	Extracellular, immunoglobulin domain 1	Enhances CD147 activity	45
Monocarboxylate transporters	Transmembrane, E218	Facilitates MCT surface expression	23
CD98, β1-Integrins	Extracellular	Induces homotypic cell aggregation	22
MMPs	Extracellular	Induces the production of secreted MMPs	29
Caveolin-1	Extracellular, immunoglobulin domain 2	Inhibits CD147 dimerization and activity	28
CyPA	Extracellular, P180	Induces intracellular signalling events and chemotaxis	71
CyPB	Extracellular	Induces intracellular signalling events and adhesion to matrix	84,85
CyP60	Transmembrane, P211	Stimulates CD147 surface expression	98

responsible for many interactions of CD147 with its partners (Table 1).

The network of CD147-interacting molecules is not fully known yet and is likely to grow. Some of these interactions, such as CD147 oligomerization,[18] binding to integrins,[19] CD98[20–22] or to the proton-coupled transporters of monocarboxylates (MCT-1 and MCT-4[23–27]) are well characterized both biochemically and functionally. Others, like CD147 interactions with caveolin-1,[28] MMP-1[29] or carbohydrates[30] are less understood and await further analysis. Despite this large number of partners (or because of it), the role of CD147 in normal cell physiology is not fully understood. Several lessons have been learned from studying CD147$^{-/-}$ knock-out mice. These animals are defective in MMP regulation (see below), lymphocyte responsiveness,[31] spermatogenesis[32] and neurological functions at the early stages of development.[33] Female mice deficient in CD147 are infertile because of the failure of female reproductive processes, including not only implantation but also fertilization.[34] CD147-deficient animals also show severe reduction in accumulation of the monocarboxylate transporter (MCT)-1 and -3 proteins in the retinal pigment epithelium, supporting a proposed role for CD147 in targeting these transporters to the plasma membrane.[26] Interestingly, positioning of the retinal lactate transporters appears to be regulated by the rare 3-immunoglobulin-like domain form of CD147, as cDNAs for this form have been identified in human and mouse retina.[16] In addition, CD147 knockout mice are characterized by enhanced mixed lymphocyte responses[31] indicating a potential negative regulatory function of CD147 in T-cell regulation. A recent study suggested that CD147 on T lymphocytes sends negative regulating signals via modification of glycosylphosphatidylinositol microdomains.[35] Still, the best-characterized functions of CD147 are stimulation of MMP production and adhesion.

The MMP-stimulating activity of CD147 has been recently reviewed.[36] Matrix metalloproteinases (MMPs) are key enzymes in maintaining integrity of the extracellular matrix which acts as both a structural scaffold for cells in a tissue and a medium for the cell–cell communications. CD147 on tumour cells was shown to stimulate the production by fibroblasts of MMP-1 (collagenase), MMP-2 (gelatinase) and MMP-3 (stromelysin-1), but not tissue inhibitor of metalloproteinase (TIMP)-1 or TIMP-2.[37,38] The synthesis of two out of three membrane-type MMPs (MT1-MMP, MT2-MMP, but not MT3-MMP) is also facilitated by CD147 in the human glioblastoma/brain-tumour derived fibroblast and human melanoma/dermal fibroblast cocultures.[39,40] The mechanism of such stimulation is not fully understood, but involves phosphorylation of the p38 mitogen-activated protein kinase[41] and depends, at least partially, on N-glycosylation of the CD147 extracellular domain.[42]

Recent studies show that overexpression of CD147 in fibroblasts[43] or in tumour cells[44] also facilitates the MMP production within the same cell culture. It has been suggested that the transmembrane portion and the glycosylation status of the extracellular domain of CD147 may regulate this process.[43,45] CD147$^{-/-}$ knock-out mice are defective in implantation, which may indicate a misregulation of MMP production.[46]

A role for CD147 in cellular adhesion is suggested by its involvement in the blood–brain barrier[5,47] and interaction with such adhesion molecules as integrins α3β1 and α6β1.[19,22] Indeed, recent data demonstrate that CD147 expressed on erythrocytes acts as an adhesion molecule, which apparently plays a critical role in the recirculation of mature erythrocytes from the spleen into the general circulation.[48] Masking of CD147 on the surface of erythrocytes leads to their selective trapping in the spleen, induction of an anaemia, and consequently, *de novo* erythropoietin-mediated erythropoiesis in this organ and in the bone marrow. In murine cerebral endothelial cells, expression of CD147 on the cell surface is coupled with expression of the intercellular adhesion molecule-1 (ICAM-1) upon infection with mouse hepatitis virus 4.[49] Moreover, CD147

monoclonal antibodies induce homotypic cell aggregation of monocytic cell line U937 through the leucocyte function-associated (LFA)-1/ICAM-1 pathway[50].

CD147 in disease

The role of CD147 in tumour biology has been the topic of many studies. CD147 stimulates MMP production in stromal fibroblasts and endothelial cells around the tumour, as well as in tumour cells themselves, by a mechanism involving homophilic interactions between CD147 molecules on apposing cells after membrane vesicle shedding.[51,52] Elevated CD147 levels were detected in numerous malignant tumours and have been correlated with tumour progression in experimental and clinical conditions. In addition, CD147 stimulates angiogenesis via induction of vascular endothelial cell growth factor,[53] invasiveness via stimulation of MMP[44] and multidrug resistance through hyaluronan-mediated up-regulation of ErbB2 signalling and cell survival pathway activities.[54,55] In human hepatoma cells, CD147 was shown to regulate calcium entry by NO/cGMP pathway and, subsequently, govern the progression of metastasis.[13] Recently, CD147 has been proposed as a novel marker of poor outcome in serous ovarian carcinoma.[56] In support of its key role in the processes of tumorigenesis, CD147 was reported as one of the most constantly up-regulated mRNAs in metastatic cells.[57]

CD147 has been implicated also in many other pathological processes. Its up-regulation in tissues has been identified in both malignant and non-malignant conditions, including experimental ventilator-induced lung injury,[58] rheumatoid arthritis (RA),[59,60] chronic liver disease induced by hepatitis C virus,[61] heart failure,[62,63] ischaemic myocardial injury[64] and atherosclerosis.[65] CD147 level is increased in smokers' bronchoalveolar lavage fluid.[66] It is important to note that treatment of acute graft-versus-host disease patients with anti-CD147 antibody was shown to be very effective, in part due to decreased leucocyte activation.[67] CD147 might represent a universal coreceptor for viral entry into the host cell, as it was shown to enhance infection by HIV-1 and coronavirus.[68,69] A recent report identified CD147 as an integral part of the multiprotein γ-secretase complex that cleaves the β-amyloid precursor protein to produce amyloid β-peptides associated with the formation of amyloid plaques in Alzheimer's disease patients.[70] The molecular mechanisms responsible for this activity of CD147 are not known and await more in-depth analysis.

CD147–cyclophilin interactions

Identification of CD147 as a signalling receptor for extracellular cyclophilins[71] added a new dimension to biological properties of this interesting molecule. CD147 was picked up in our search for the receptor responsible for the extracellular activity of cyclophilin A (CyPA). CyPA is a ubiquitously expressed intracellular protein belonging to the immunophilin family[72] and is best known as the principal ligand for the potent immunosuppressive drug, cyclosporin (CsA).[73–75] CyPA also possesses peptidyl-prolyl *cis–trans* isomerase activity and is believed to play an important role in protein folding.[76] Although CyPA was initially described as a solely intracellular protein, later experiments have revealed it can be released by cells in response to inflammatory stimuli.[77,78] Indeed, the presence of elevated levels of extracellular cyclophilins has been reported in several different inflammatory diseases, including severe sepsis,[79] vascular smooth muscle cell disease[80] and RA.[81] In the case of RA, levels of extracellular CyPA within synovial fluid of patients with ongoing disease were found to directly correlate with neutrophil numbers present in the same fluid, suggesting an association between cyclophilin levels and disease severity.[81] In a recent study, cartilage chondrocytes were shown to secrete cyclophilin B (CyPB) in response to matrix metalloproteinases, providing an additional source of extracellular cyclophilins released during ongoing RA.[82]

One way in which these extracellular cyclophilins might contribute to inflammatory responses is via their chemotactic properties.[83] Several studies have demonstrated a chemotactic activity of extracellular CyPA and CyPB for neutrophils, eosinophils, and T lymphocytes[77,78,84] suggesting the presence of a cyclophilin receptor on target cells. A yeast two-hybrid screen with a B-cell cDNA library for cell-surface CyPA-binding molecules identified CD147 as a potential candidate, and subsequent analysis demonstrated that CD147 mediates the signalling and chemotactic activities of CyPA.[71] The signalling initiated by cyclophilin B also has been found to require CD147[85] suggesting that CD147 is the principal signalling receptor for extracellular cyclophilins. In support of this notion, Allain and colleagues[84] demonstrated a strong inhibitory effect of anti-CD147 antibody on CyPB-mediated adhesion of lymphocytes to fibronectin, a process dependent on CyPB-induced signalling.

While CD147 appears to be essential for cyclophilin-dependent signalling related to chemotaxis and adhesion of immune cells, a recent study by Yang and colleagues[86] demonstrated that CyPA-induced smooth muscle cell (SMC) proliferation is not blocked by anti-CD147 antibody. Interestingly, extracellular CyPA affected transcription of several genes, including CD147, in SMCs and pancreatic cancer cells,[86,87] and it would be important to determine whether this activity can be blocked by anti-CD147 antibody. It is possible that cell proliferation-stimulating activity of CyPA is mediated by a distinct receptor and can be observed only in cells that express this receptor.

Of note, both the signalling and chemotactic activities of CyPA and CyPB are also dependent on the presence of heparan sulphate proteoglycans (HSPGs), which likely serve as

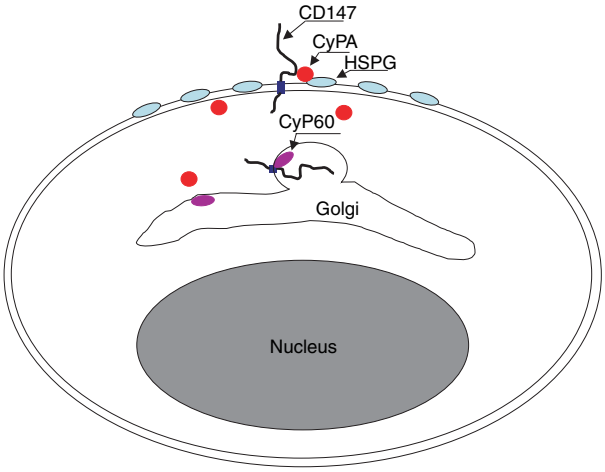

Figure 1. Proposed interactions between cyclophilins and CD147. CD147 is transported to the cell surface via the Golgi network. Interaction between cyclophilin 60 (CyP60) and Pro211 (the residue at the interface between the transmembrane and extracellular domains of CD147) occurs in the lumen of Golgi vesicle. Cyclophilin A (CyPA) is excluded from Golgi and does not have access to Pro211. Extracellular CyPA interacts with cell surface heparan sulfate proteoglycan (HSPG) and Pro180 in the extracellular domain of CD147. Membrane-proximal Pro211 is not accessible for interaction for sterical reasons. Not drawn to scale.

primary binding sites for these cyclophilins on target cells (Fig. 1).[71,82] Removal of HSPGs from the cell surface of neutrophils eliminates signalling responses to cyclophilins and abolishes cyclophilin-dependent chemotaxis and adhesion of neutrophils and T cells.[71,84] Interestingly, activation of T cells removes the dependence of CyPA-specific responses on heparans (Constant and Bukrinsky, unpublished observations), suggesting that increased CD147 expression and dimerization in activated T cells enhance CyPA binding to CD147. This result supports the model whereby cyclophilin binding to HSPGs is required to complement low-affinity interaction between cyclophilins and CD147 expressed on non-activated cells. Binding of extracellular CyPA to HSPGs may preclude its interaction with Pro211 (Fig. 1), which would result in a more stable binding illustrated by CyP60–CD147 interaction (see below).

The proline 180 and glycine 181 residues in the extracellular domain of CD147 were found to be critical for both signalling and chemotactic activities induced by extracellular CyPA.[71] Also crucial were active site residues of CyPA, because peptidyl-prolyl *cis-trans* isomerase-defective CyPA mutants failed to initiate signalling events.[71] These results suggest an unusual, rotamase-dependent mechanism of signalling through this receptor. Such a mode of signalling requires only a transient interaction between the ligand and the receptor and is consistent with low-affinity binding interaction between CD147 and CyPA.[71]

While the chemotactic properties of cyclophilin–CD147 interactions have been well documented *in vitro*, little is known of the physiological and pathological relevance of such interactions *in vivo*. Recently, we reported that anti-CD147 antibodies, as well as non-immunosuppressive CsA analogue, SDZNIM811, significantly reduced the inflammatory response in a mouse model of acute lung inflammation that closely resembles acute lung injury in humans.[88] Strikingly, treatment of the lipopolysaccharide-sensitized mice with anti-CD147 monoclonal antibody (mAb) or SDZNIM811 led to a 40–50% inhibition of the development of neutrophilia within lung tissues and airways. Importantly, treating mice with a combination of anti-CD147 and CsA led to an inhibition of neutrophil infiltration only slightly greater than that induced by the individual treatments, indicating that anti-CD147 and CsA are probably acting on the same cyclophilin–CD147 interactions. This result reduces the possibility that other activities of CD147, such as an adhesion molecule,[48] contribute to the observed effects of the anti-CD147 antibody on neutrophil recruitment. In more recent studies, we have investigated the impact of blocking cyclophilin–CD147 interactions in mouse models of allergic asthma and RA. Preliminary results suggest that mice treated with anti-CD147 mAb at the onset of asthma-mediated responses have >50% reduction in lung eosinophilia and airway hyperresponsiveness (Constant and Bukrinsky, unpublished observations). In the case of RA, we observed $\geq$75% reduction in joint inflammation following anti-CD147 treatment of mice with collagen-induced arthritis (Constant and Bukrinsky, unpublished observations). This finding is consistent with a recent report by Zhu and coauthors[89] who demonstrated increased expression of CD147 on monocytes/macrophages in RA and found that chemotaxis of monocytes to synovial fluid from RA patients can be blocked by anti-CD147 antibody or CD147 antagonistic peptide. Taken together, these results suggest an important contribution of cyclophilin–CD147 interactions to the initiation and/or progression of inflammatory responses, via recruitment of leucocytes into inflamed tissues. Moreover, the demonstration that these interactions play a direct role in different types of inflammatory conditions, provides an attractive new target for intervention.

The capacity of CD147 to serve as a signalling receptor for CyPA also forms the basis of its activity in human immunodeficiency virus-1 (HIV-1) infection.[68] Indeed, CD147 did not enhance infection of CyPA-deficient HIV-1. It is possible that some of other known activities of CD147 are mediated by its interaction with cyclophilins.

Regulation of the cell surface expression of CD147

The fact that CD147 interacts with extracellular cyclophilins suggests that such an interaction may also occur within a cell, where cyclophilins are abundant. One of

the well-established activities of cyclophilins is regulation of protein trafficking in cells. CyPA, for instance, has been shown to be a component of the transport complexes that regulate surface expression of asialoglycoprotein receptor expression in human cells.[90] In yeast, CyPA was shown to mediate the import of fructose-1,6-bisphosphatase into intermediate transport vesicles for vacuole delivery[91] and to facilitate nuclear export of Zpr1, an essential zinc finger protein.[92] Nina A (cyclophilin A homologue in *Drosophila*) is a key component of the rhodopsin trafficking.[93,94] In all these examples, except Zpr1, cyclophilins were found to form a specific stable complex with the substrate, suggesting that they function as a chaperone escorting its protein substrate through the traffic pathway. Recent reports[95,96] demonstrate that cyclophilins may be involved in cell surface externalization of two other proteins, namely insulin receptor and Flt3 ligand. In both cases, treatment of the cells with CsA reduced surface expression of the proteins without altering their total cellular levels, suggesting a block at the step of transition from the endoplasmic reticulum to the trans-Golgi network. The cyclophilin(s) involved in regulation of trafficking of these proteins and the cyclophilin-interacting domains within these targets have not yet been identified.

Analysis of CD147 cell-surface expression revealed its unexpected sensitivity to CsA, suggesting involvement of a cyclophilin in the regulation of CD147 intracellular trafficking.[97] Solution binding experiments demonstrated the role of CD147 transmembrane domain as a main binding site for CyPA and localized this interaction to a proline-containing peptide in the transmembrane domain. Mutation of this proline residue (Pro211) diminished transport of CD147 to the plasma membrane without reducing the total level of CD147 expression.[97]

The cyclophilin involved in CD147 expression was recently identified as cyclophilin 60 (CyP60), a distinct member of the cyclophilin family of proteins.[98] CD147 coimmunoprecipitated with CyP60, and confocal immunofluorescent microscopy revealed intracellular colocalization of CyP60 and CD147 at the plasma membrane, consistent with CyP60 functioning as a chaperone for CD147. These results suggest that CyP60 plays an important role in the translocation of CD147 to the cell surface. Given that CyP60 interacts with Pro211 located at the interface of the membrane and extracellular domains of CD147.[97] CyP60 must localize to the lumen of the Golgi apparatus to get access to this site on CD147 (Fig. 1). The more abundant CyPA is excluded from the Golgi lumen (Fig. 1), explaining lack of intracellular interaction between CyPA and CD147.[98] It will be interesting to determine whether CyP60 interaction with CD147 also involves rotamase activity of CyP60, as has been shown for CyPA–CD147 interaction.[71] However, stability of this interaction illustrated by coimmunoprecipitation of CyP60 and CD147[98] suggests involvement of some type of stable non-covalent binding.

CD147 has been shown to interact with several other proteins that may influence its localization. For example, described previously interaction of CD147 with MCT1 and MCT4, the proton-coupled transporters of monocarboxylates,[23] occurs within the cellular membrane and critically depends on the centrally positioned glutamic acid residue 218 in the CD147 transmembrane domain. When association of CD147 with monocarboxylate transporter MCT1 was disrupted by mutating this glutamic acid, neither CD147 nor MCT1 reached the plasma membrane.[27] CD147 also interacts with caveolin-1 on a cell surface and this interaction seems to negatively regulate clustering and activity of CD147.[28] Association with caveolin-1 depends on the second immunoglobulin domain in the extracellular portion of CD147. Finally, leucine 252 (along with the adjacent amino acids 243, 244, 245 and 246) in the cytoplasmic domain of CD147 was identified as a basolateral signal targeting CD147 to the basolateral membrane in extraocular epithelia. Deletion of these amino acids leads to mistargeting of CD147 to the apical membranes.[99] This signal seems to function only in some cell types (e.g. it was not recognized in human retinal pigment epithelium cells[99]), suggesting that it mediates interaction with some cell-specific regulator of protein trafficking. Future studies will hopefully integrate these findings into a unifying model of CD147 trafficking. Such model will not only suggest new targets for therapeutic interventions in diseases where CD147 is recognized as a pathogenetic factor (e.g. cancer or rheumatoid arthritis), but will also explain the role of CD147 in other biological processes, such as development of the eye or spermatogenesis.[100,101]

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