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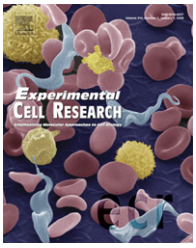

Research Article

Cyclophilin B induces integrin-mediated cell adhesion by a mechanism involving CD98-dependent activation of protein kinase C-δ and p44/42 mitogen-activated protein kinases

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ABSTRACT

Initially identified as a cyclosporin-A binding protein, cyclophilin B (CyPB) is an inflammatory mediator that induces adhesion of T lymphocytes to fibronectin, by a mechanism dependent on CD147 and α4β1 integrins. Recent findings have suggested that another cell membrane protein, CD98, may cooperate with CD147 to regulate β1 integrin functions. Based on these functional relationships, we examined the contribution of CD98 in the pro-adhesive activity of CyPB, by utilizing the responsive promonocyte cell line THP-1. We demonstrated that cross-linking CD98 with CD98-AHN-18 antibody mimicked the responses induced by CyPB, *i.e.* homotypic aggregation, integrin-mediated adhesion to fibronectin and activation of p44/42 MAPK. Consistent with previous data, immunoprecipitation confirmed the existence of a heterocomplex wherein CD147, CD98 and β1 integrins were associated. We then demonstrated that CyPB-induced cell adhesion and p44/42 MAPK activation were dependent on the participation of phosphoinositide 3-kinase and subsequent activation of protein kinase C-δ. Finally, silencing the expression of CD98 by RNA interference potently reduced CyPB-induced cell responses, thus confirming the role of CD98 in the pro-adhesive activity of CyPB. Altogether, our results support a model whereby CyPB induces integrin-mediated adhesion *via* interaction with a multimolecular unit formed by the association between CD147, CD98 and β1 integrins.

Introduction

First identified as the main binding proteins for cyclosporin A (CsA), cyclophilins are ubiquitously distributed peptidyl-prolyl *cis-trans* isomerases (PPIase), for which most of the studies concerning their biology have focused on their intracellular functions [1,2]. However, accumulating data have indicated a key role for secreted cyclophilins A (CyPA) and B (CyPB) as regulators of inflammation. The expression of these proteins is increased as a response to inflammatory stimuli and oxidative stress [3–7]. Both CyPA and CyPB trigger chemotaxis of polymorphonuclear leukocytes, monocytes and T lymphocytes, supporting the hypothesis that they may contribute to the guidance of leukocyte populations to inflammatory sites [3,6–10]. We and others have demonstrated that the activities of secreted CyPA and CyPB are related to interactions with CD147 [9–11]. By using site-directed mutants of CD147, Yurchenko *et al.* [12] demonstrated that a proline residue located in an exposed loop of CD147 was crucial for CyPA to induce cell chemotaxis. Consistent with these findings, we also demonstrated that the central

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core of CyPB, which contains CsA binding domain and catalytic site, is required for efficient binding to T lymphocytes. Moreover, a PPIase defective mutant was found unable to trigger p44/42 mitogen-activated protein kinases (MAPK) activation and subsequent integrin-mediated adhesion of T cells to fibronectin [13,14]. These data suggest that CyPA and CyPB trigger common biological responses by a mechanism that involves prolyl isomerization of CD147, making this protein as an essential component of the membrane receptor of extracellular cyclophilins.

CD147 is a 50-60 kDa-type I transmembrane protein also known as extracellular matrix metalloproteinase inducer (EMMPRIN) or basigin. CD147 is broadly expressed on human peripheral blood cells, endothelial cells and cultured cells, and is involved in reproduction, neural function, inflammation, tumour invasion and human immunodeficiency virus-1 (HIV-1) infection, functioning either as a signal transmitting molecule or directly as a regulator of adhesion. CD147 was proven to be responsible for the induction of expression of several matrix metalloproteinases [15,16], and to enable insertion of monocarboxylate transporters (MCT)-1 and -4 in the membrane, which facilitates import and/or export of lactate and pyruvate [17]. In addition to these functions, accumulating data have suggested that CD147 may interact with integrins. Antibodies to CD147 were reported to modulate homotypic cell aggregation [18,19] and CD147 was found to co-immunoprecipitate with β1 integrins and to colocalize with these integrins in the areas of cell-cell contacts [20].

Although it is not clear yet as how these molecules share their roles in cell adhesion, recent findings suggest that another cell surface protein, CD98, may functionally cooperate with CD147 to regulate β1 integrin functions [19]. Consistent with these findings, combination of covalent cross-linking, mass spectrometric protein identification and co-immunoprecipitation experiments revealed the existence of a cell surface multimeric complex, where CD147 and CD98 association plays a central organizing role [17]. CD98, also known as 4F2, is an 85 kDa-type II integral membrane protein initially characterized as a T cell activation marker [21]. It has been subsequently found covently linked to one of at least six alternative 40 kDa-light chains, four of which have been identified as members of heterodimeric amino acid transporter family (HAT). The function of amino acid transporters is determined by the light chain, while the heavy chain is required for the functional cell surface expression and localization of the HAT complex [22,23]. As CD147, a striking feature of CD98 is the large diversity of functions in which it has been implicated, *e.g.* haematopoietic cell differentiation and functions, cell growth and fusion, and virus infection. As examples, CD98 has been reported to regulate osteoclast differentiation and T-lymphocyte stimulation [24,25]. The ability of antigen-presenting cells to deliver co-stimulatory signals to T lymphocytes cells could be blocked by antibodies to CD98 [26]. Antibodies against CD98 also inhibit the formation of cell syncytia by HIV and other viruses [27,28]. RNA interference-induced reduction in CD98 expression suppresses cell fusion of human placental cells [29]. In addition to these activities, CD98 appears to play a key role in the regulation of integrin functions. The first data came from studies showing that CD98 enhanced integrin-dependent cell aggregation and fusion [19,30]. Physical interaction between CD98 and β1 integrins have been then demonstrated [31,32] and CD98 was reported to co-immunoprecipitate with β1 integrins in a number of different cell lines [33–35] and peripheral blood T lymphocytes [36]. Furthermore, evidence from a number of studies supports a model where CD98 regulates β1 integrin affinity, because cross-linking of CD98 with antibody leads directly to the activation of β1 integrins [25,35–37].

Based on the functional relationships between CD147, CD98 and β1 integrins, we thought the idea that CD98 might coordinate the responses triggered by CyPB and regulate integrin-mediated adhesion of responsive cells. In this study, we have thus examined the contribution of CD98 in CyPB-induced cell adhesion to fibronectin. To this end, we have used the responsive THP-1 promonocytic cell line [14]. Consistent with the previously published data, we first confirmed that CD147, CD98 and β1 integrins are associated within a functional multimolecular unit. Then, we demonstrated that the pro-adhesive activity of CyPB is dependent on the activation of protein kinase C-δ (PKCδ), a mechanism in which CD98 plays a pivotal role.

Materials and methods

Antibodies and reagents

Recombinant human CyPB was produced and purified as described [38]. CyPB was detoxified on Detoxi-Gel Endotoxin Removing Gel (Pierce Chemicals, Rockford, IL, U.S.A). The endotoxin content of the preparation was less than 0.09 E.U./μg, as determined by the chromogenic *Limulus* lysate assay (Biowhittaker, Walkersville, MD, USA). Human fibronectin was a gift from Dr. P. Delannoy (University of Lille, France). Mouse monoclonal antibodies to CD147 (clone HIM6), to β1 integrin (clone 18) and to PKCδ (clone 14) were purchased from BD Pharmingen (San Diego, CA, USA). Mouse monoclonal antibodies to CD147 (clone MEM-M6/1), to CD98 (clone 4F2), to the phosphorylated forms of ERK1/2 (p44/42 MAPK) (E-4) and goat polyclonal antibodies to CD98 were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse monoclonal antibody to human CD14 (My4) and rabbit polyclonal anti-phospho-PKCδ (Ser643) were from Beckman Coulter (Fullerton, CA, USA) and Cell Signaling (Beverly, MA, USA), respectively. Mouse monoclonal antibodies to CD98 (clone AHN-18) and to β1 integrins (clone MAB2253) were from Chemicon International (Temecula, CA, USA). These antibodies were recommended as inducer of cell homotypic aggregation and efficient agent of immunoprecipitation, respectively. The blocking monoclonal mouse antibodies to integrin subunits (anti-α2, -α4, -α5, -β1, -β2,) were from Calbiochem (La Jolla, CA, USA). Rabbit polyclonal anti-ERK1/2, mouse monoclonal anti-CD44 (clone A3D8), fluorescein-conjugated antibodies to mouse and goat IgG, horseradish peroxidase-conjugated anti-goat IgG, and isotype-matched control antibodies were from Sigma Chemicals Co (St Louis, MO, USA). Horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG antibodies were from Amersham Pharmacia Biotech (Little Chalfont Buckinghamshire, UK). PD98059, rottlerin and 1,25-dihydroxy-vitamine D3 were from Calbiochem (La Jolla, CA, USA), and wortmannin from Alexis Corp. (San Diego, CA, USA). The thiol cleavable cross-linker 3, 3′-dithiobis[sulfosuccinimidylpropionate] (DTSSP) was from Pierce. Cell culture products were from Cambrex (Walkersville, MA, USA). Oligonucleotide PCR primers and siRNA duplexes were synthesized and purified