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CD147 Is a Signaling Receptor for Cyclophilin B

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Cyclophilins A and B (CyPA and CyPB) are cyclosporin A binding proteins that can be secreted in response to inflammatory stimuli. We recently identified CD147 as a cell-surface receptor for CyPA and demonstrated that CD147 is an essential component in the CyPA-initiated signaling cascade that culminates in ERK activation and chemotaxis. Here we demonstrate that CD147 also serves as a receptor for CyPB. CyPB induced Ca^{2+} flux and chemotaxis of CD147-transfected, but not control, CHO cells, and the chemotactic response of primary human neutrophils to CyPB was blocked by antibodies to CD147. These results suggest that CD147 serves as a receptor for extracellular cyclophilins. © 2001 Academic Press

Cyclophilins A and B (CyPA and CyPB) belong to the immunophilin family of peptidyl-prolyl *cis–trans* isomerases (1) and were recognized as the host cell receptors for the potent immunosuppressive drug, cyclosporin A (2, 3). While CyPA is predominantly cytosolic [but can be secreted by macrophages in response to stimulation with bacterial endotoxin (4) or by vascular smooth muscle cells in response to oxidative stress (5)], CyPB is found within the endoplasmic reticulum and is secreted into milk and plasma (6–8). Both CyPA and CyPB are believed to be involved in inflammatory reactions, as their levels in biological fluids often correlate with inflammation, e.g., in rheumatoid arthritis (9) or sepsis (10). Secreted CyPA can stimulate signaling response in target cells (5), is a potent neutrophil (4) and eosinophil (11) chemoattractant *in vitro,* and elicits an inflammatory response characterized by a rapid influx of neutrophils when injected *in vivo* (4, 11). CyPB, on the other hand, was shown to enhance platelet adhesion to collagen (12). All these activities suggest the existence of a specific receptor on responsive cells.

Recently, we identified CD147 as a receptor for extracellular (13). Given that CyPA and CyPB are closely related (CyPB family is distinguished from the CyPA family by the presence of endoplasmic reticulum-directed signal sequence) and have very similar structure (14), it seemed probable that CD147 could also serve as a receptor for CyPB. In this report we provide a formal proof of this hypothesis. We demonstrate that expression of CD147 is sufficient to make CHO cells responsive to stimulation with CyPB, and that antibodies to CD147 inhibit CyPB-induced neutrophil chemotaxis.

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MATERIALS AND METHODS

Cells and reagents. The CHO-K1 cell was purchased from ATCC. The antibodies to p42/44 MAP kinase were from New England Biolabs (Beverly, MA); anti-CD147 monoclonal antibodies were purchased from Ancell (Bayport, MN).

Plasmids. Human CyPA and CyPB were cloned into the pET14b vector (Novagen, Madison, WI) and purified using His-Bind methodology (Novagen), essentially as described previously (15).

Calcium mobilization assay. Mobilization of intracellular calcium in CyPA–stimulated cells was assessed as described elsewhere (15). Briefly, 0.6 ml of Fura-2-AM loaded cells (5×10^6 cells/ml) was stimulated with 100–500 nM human CyPA per sample, and fluorescence emission at 340 and 380 nm was measured on a Perkin–Elmer LS50B luminescence spectrometer.

Analysis of ERK activation by Western blotting. Serum-starved cells were treated with 25 nM of CyPB. Cell lysates were separated on 10% SDS–PAGE and subjected to Western blotting analysis using antibodies specific for the nonphosphorylated and phosphorylated forms of Erk1/Erk2 MAP kinases following the protocol recommended by the manufacturer (New England Biolabs).

Chemotaxis assays. Neutrophils were obtained from heparinized venous blood by Ficoll–Hypaque gradient centrifugation, as described (4). Neutrophil chemotaxis was assessed in a 48-well modified Boyden chamber (16) with the two compartments separated by a polyvinylpyrrolidone (PVP)-free polycarbonate filter with a 5-μm pore size (Whatman Nuclepore, Tewksbury, MA). Neutrophils (1.5×10^6 cells/ml) in Gey's balanced salts solution supplemented with 2% bovine serum albumin and 20 mM Hepes (GBSA) were added to the compartment above the filter, and test samples of possible chemoat-

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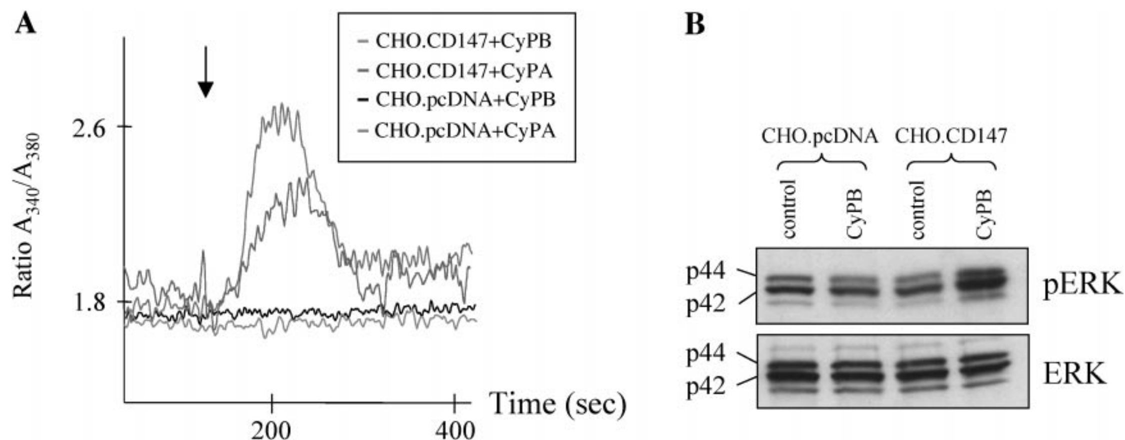

FIG. 1. CD147 mediates CyPB-specific signaling events. (A) CyPB induces Ca^{2+} flux in CD147-expressing cells. CHO.pcDNA or CHO.CD147 cells were loaded with Fura-2-AM, stimulated with recombinant human CyPA (50 nM) or CyPB (25 nM) and analyzed on a luminometer. The arrow indicates the time of stimulation. Results are shown for one representative experiment out of three performed. (B) CyPB stimulates ERKs phosphorylation. Serum-starved CHO.CD147 or CHO.pcDNA cells were stimulated or not (control) with CyPB (25 nM), lysed and analyzed by Western blotting using anti-ERK (bottom panel) or anti-phosphoERK (upper panel) monoclonal antibodies.

tractants diluted in chemotaxis medium were present below the filters. Chambers were incubated at 37°C and 5% CO_2 for 30 min; then the filters were recovered, fixed, and stained with Giemsa reagent. The number of cells appearing on the lower face of the filter was recorded in four high-power fields for each well, and each experimental condition was assayed in triplicate wells. *N*-Formyl-Met-Leu-Phe (FMLP) (10^{-7} M) was used as a positive control.

CHO.pcDNA and CHO.CD147 cells were assayed for chemotaxis in a manner identical to that described for neutrophil chemotaxis except that the two wells were separated by a 10-μm pore size filter (Neuro Probe, Inc., Gaithersburg, MD) precoated with fibronectin (10 μg/ml fibronectin for 2 h at 37°C and 5% CO_2), and the chambers were incubated at 37°C and 5% CO_2 for 4.5 h.

RESULTS

To determine whether CyPB can initiate intracellular signaling events in CD147-expressing cells, we measured Ca^{2+} flux in CHO.CD147 and CHO.pcDNA cells stimulated with CyPB. A characteristic flux of intracellular Ca^{2+} was observed in CHO.CD147, but not in CHO.pcDNA cells (Fig. 1A). Interestingly, CyPB appeared to induce Ca^{2+} response more efficiently than CyPA, since the optimal response to CyPB was stronger and was obtained at a lower concentration of the ligand (25 nM for CyPB versus 50 nM for CyPA) (Fig. 1A).

To investigate other signaling pathways that might be initiated by CyPB through CD147, we analyzed the phosphorylation status of ERK MAP kinases in CyPB-treated CHO.CD147 and CHO.pcDNA cells. A characteristic increase in the level of phosphorylated ERKs was observed after the addition of CyPB to CHO. CD147, but not to CHO.pcDNA cells (Fig. 1B, upper panel). The amount of total (phosphorylated and nonphosphorylated forms) ERKs was not changed by CyPB (Fig. 1B, bottom panel), indicating that CyPB stimulated phosphorylation and activation of the preexisting protein.

Our recent work demonstrated that CD147-mediated signaling response culminates in chemotaxis of CyPA-treated cells (V.Y., Gabriele Zybarth, M.O'C., W.W.D., Giovanni Franchin, Tang Hao, H.G., B.T., B.S., and M.B., manuscript submitted). However, chemotactic activity has not been described for CyPB. We thus investigated whether CyPB could induce chemotaxis of cells previously shown to respond to CyPA stimulation, i.e., CD147-transfected CHO cells and primary neutrophils. Using modified Boyden chambers, we first analyzed chemotaxis of CHO cells engineered to express CD147 (CHO.CD147). Results presented in Fig. 2A demonstrate that CyPB is chemotactic for CHO.CD147, but not for control CHO.pcDNA cells. Similarly, CyPB induced neutrophil chemotaxis (Fig. 2B). This chemotactic response was inhibited by antibodies to CD147, but not by isotype-matched control antibodies. To exclude a nonspecific activity of the anti-CD147 antibody, we tested its effect on FMLP-induced chemotaxis, which is mediated by a different receptor (17). No decrease in chemotaxis was observed in that experiment, indicating that the antibody specifically inhibited CD147-mediated chemotactic response to CyPB.

DISCUSSION

The experiments presented in this report demonstrate that CD147 mediates the cellular responses to CyPB. This finding suggests that CD147 might represent the type I CyPB-binding site identified in previous studies as the signaling receptor for CyPB (18). Those studies also demonstrated interaction of CyPB with sulfated glycosaminoglycans (heparans) (CyPB-binding site type II), which enhanced signaling by CyPB, presumably by concentrating the ligand and

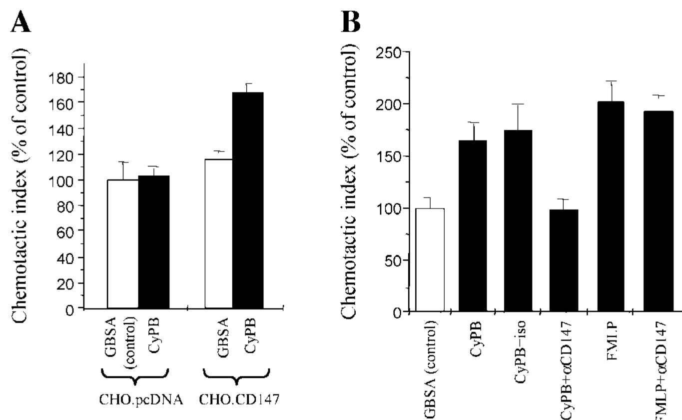

FIG. 2. CD147 mediates chemotactic response to CyPB. (A) CyPB induces chemotaxis of CHO.CD147 cells. CyPB (1.5 nM) or BSA in Gey's solution (GBSA) was added to the bottom chamber of the Boyden chamber assembly, while the upper chamber contained CHO.pcDNA or CHO.CD147 cells. Chemotaxis was measured as the number of cells migrating to the bottom side of the filter separating the chambers and is presented relative to GBSA-specific chemotaxis of CHO.pcDNA. Data are presented as means of three independent wells ±SD. One representative experiment out of two performed is shown. (B) CD147 mediates chemotactic response of neutrophils to CyPB. Experiment was performed as in A, except that primary human neutrophils isolated from peripheral blood were used. Chemotactic peptide *N*-Formyl-Met-Leu-Phe (FMLP) was used at 100 nM as a positive control. Anti-CD147 monoclonal antibody (αCD147) or isotype-matched control antibodies were used at 50 μg/ml. Results are for one representative experiment of two performed.

presenting it to the receptor. While in this work we did not investigate the role of heparans on CyPB-induced signaling through CD147, our recent study (V.Y., Gabriele Zybarth, M.O'C., W.W.D., Giovanni Franchin, Tang Hao, H.G., B.T., B.S., and M.B., manuscript submitted) demonstrated that heparans are required for signaling and chemotactic activity of CyPA. It seems probable that CyPB-induced signaling through CD147 also needs participation of heparans. Interestingly, an earlier report suggested that CyPA does not compete with cell surface-bound radiolabeled CyPB (18). Since cell binding of both CyPA and CyPB is mostly determined by heparans, which are extremely abundant on a cell surface, it appears that CyPA and CyPB use different classes of heparan molecules for binding, or that the affinity of CyPA for heparans is lower than that of CyPB. These differences might be responsible for higher signaling potency of CyPB (Fig. 1A) and might also contribute to specificity of the cellular responses to stimulation by cyclophilins.

In summary, this report demonstrates that CD147 acts as a signaling receptor for CyPB. Future studies will determine the physiological and pathophysiological role of CyPB–CD147 interaction.

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