THESIS PROPOSAL

Identification and characterization of functional partners of the CD147 molecule

Assigned Program: Inflammation and Immunology

Location: Institute of Immunology, VIRCC, University of Vienna, Brunner Strasse 59, A-1235 Vienna

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Summary and aim

There are many types of molecules on the surface of leukocytes. Some of them have been identified and characterized, but a number are still awaiting their characterization. With the development of monoclonal antibodies which enable the identification and isolation of individual leukocyte cell surface molecules as well as the advent of molecular cloning and expressing techniques, rapid progress in understanding of the function of the less characterized molecules is now possible. This study will concentrate on the characterization of protein complexes containing CD147. CD147 is a type I integral membrane protein of the immunoglobulin superfamily. The transmembrane part of this molecule contains potential protein-protein interactions motifs: a charged residue, a glutamic acid, and a mini-leucine-zipper motif. This region is highly conserved between different species indicating a functional role, perhaps in the interaction with other proteins within the plasma membrane. The aim in this study is to screen for CD147 molecule associated proteins. To identify possible associated proteins, the yeast-two-hybrid system and co-immunoprecipitation plus the Matrix-Assisted Laser Desorption/Ionization (MALDI) technique will be performed. Finally, the functional consequence of these interactions will be proved in human cells.

Background

The human leukocyte surface molecule CD147 was assigned at the Sixth International Workshop on Human Leukocyte Differentiation Antigen (1). CD147 is also known as M6 antigen (2), human basigin (3), or extracellular matrix metalloproteinase inducer (EMMPRIN) (4). Comparison of the CD147 sequence with other molecules indicates that it is the species homologue of the rat protein OX-47/CE9 (5), the chicken blood-brain barrier-related molecule HT7/neurothelin/5A11 (6), the mouse molecule termed gp42 (7) or basigin (8) and the rabbit homologue (9). The human gene encodes 296 amino acid residues that have a typical feature of a type I integral membrane protein of the immunoglobulin superfamily. The transmembrane part is interrupted by a charged residue, a glutamic acid, and a mini-leucine-zipper motif; both are potential protein-protein interaction motifs (2). This region is highly conserved between different species indicating a functional role, perhaps in the interaction with other proteins within the plasma membrane for signal transduction. CD147 is broadly expressed on human peripheral blood cells, endothelial cells, and cultures of hematopoietic and non-hematopoietic cells.

The full function of CD147 is still unclear. However, several studies indicate that CD147 is strongly involved in regulation of cell adhesion. Firstly, CD147 was described as a tumor cell-derived collagenase stimulatory factor (TCSF) that stimulates the expression of collagenase by fibroblasts (1). Monoclonal antibodies (mAbs) against CD147 could inhibit homotypic aggregation of the estrogen-dependent breast cancer cell line, MFC-7 and its adhesion to type IV collagen, fibronectin ad laminin (1). Recently it was found that CD147 co-precipitated with $\alpha_3\beta_1$ and $\alpha_6\beta_1$integrins, and co-localized with these integrins in areas of cell-cell contact (10). Moreover, mAbs to CD147 are potent inhibitors of both aggregation and protein tyrosine phosphorylation induced via CD98 or β_1- integrins in U937 cells (11). On the other hand, engagement of CD147 induced cell aggregation of the myeloid cell line U937 seems to involve a β_2- integrin dependent mechanism (12).

Furthermore, CD147 was found to be involved in T cell regulation. Induction of dimerization of CD147 by a mAb directed to a unique epitope resulted in strong inhibition of CD3-mediated T cell activation (13). The expression of the CD147 molecule correlated with cycling of immature thymocytes and the ligation of this molecule on immature thymocytes inhibited their development into mature T cells (14).

Operational objectives:

1st year

Aims: Identify physical interaction partners of the CD147 molecule.

First, in collaboration with partner laboratories, we will start to identify proteins associated with CD147 using the yeast-two-hybrid system. Subsequently, the physical interaction of these molecules in resting and activated human cells will be evaluated and proved or disproved.

Second, we will screen for physical interaction partners of the CD147 molecule by using co-immunopecipitation and Western blotting. The experiment will be performed using several human cell lines. Initially, we have to screen for mAbs that are useful for co-immunoprecipitation of the CD147 molecule and its partners. Furthermore, detergents have a significant role in isolation of membrane proteins. Thus, it is important to determine the appropriate detergent capable to solubilize the membrane without destroying protein-protein interactions. Different concentrations of mild detergents such as Brij-58, NP-40, deoxycholic acid and n-Dodecyl– β–D-maltoside will be used. The optimal condition of these detergents will be identified and applied as a standard method for cell lysate preparation. The various molecules co-immunoprecipitated with CD147 will be sequenced by using the MALDI technique.

2nd year

Aim: Study the functional cooperation of the CD147 molecule and its partners in the immune system.

There are several studies indicating that CD147 is strongly involved in cell adhesion and T cell regulation. Therefore, cell aggregation assays will be established with T cells to analyze the functional consequence of the interaction between CD147 and the molecules identified to be associated with it. In order to do this, the identified molecules will be over-expressed in the T cell line Jurkat using a retroviral expression system.

We found previously that a particular CD147 mAb can induce cell aggregation of both the human T cell line Jurkat and CD147 mouse transductants. Thus, using this mAb and the generated cells mention before we have an assay to study the regulatory function of the CD147 associated molecules on CD147—induced cell aggregation.

To determine, which part of the CD147 molecule and the associated molecules play a regulatory role in cell adhesion, mutation analysis will be carried out. Different mutant forms of CD147 and the interaction partners will be constructed and expressed. These forms are supposed to uncover the determinants responsible for interaction. To confirm that the induced cell aggregation is not a phenomenon of agglutination but of active signal transduction, different metabolic and cytoskeleton blocking agents will be applied.

The studies mentioned above are conducted using immortal cell lines (Jurkat). To have a better understanding of the functional role of CD147 and its partner molecules, finally, I would like to perform the functional studies in primary T cells.

Working plan

1st year

Months 1-6

- Perform yeast-two-hybrid-system
- Confirm the physical interaction of CD147 and the yeast-two-hybrid identified partners by co-immunprecipitation and Western blotting in human cells.

Months 7-12

- Screen for further interaction partners of CD147 by co-immunoprecipitation and Western blotting.
- Set the optimal condition of cell lysate preparation by using different detergents to purify the co-immunoprecipiated proteins
- Sequence the purified proteins by MALDI technique.

2nd year

Months 13-18

- Generate different transductants expressing human CD147 and its identified partner proteins.
- Perform cell adhesion assays using the generated cells.

Months 19-24

- Construct different mutation forms of CD147 and its associated molecules.
- Use the mutant forms to infect target cells.
- Establish a cell adhesion assay by using the generated cells.
- Confirm the functional studies performed in the cell line model in primary T cells.

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Timelines

	1st year		2nd year	
	Months 1-6	Months 7-12	Months 13-18	Months 19-24
Perform yeast-two-hybrid-system.				
Confirm the physical interaction of CD147 and the yeast-two-hybrid identified partners by co-immunprecipitation and Western blotting in human cells.				
Screen for further interaction partners of CD147 by co-immunoprecipitation and Western blotting.				
Set the optimal condition of cell lysate preparation by using different detergents to purify the co-immunoprecipiated proteins.				
Sequence the purified proteins by MALDI technique.				
Generate different transductants expressing human CD147 and its identified partner proteins.				
Perform cell adhesion assays using the generated cells.				
Construct different mutation forms of CD147 and its associated molecules.				
Use the mutant forms to infect target cells.				
Establish a cell adhesion assay by using the generated cells.				
Confirm the functional studies performed in the cell line model by using primary T cells.				