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CD148, a membrane protein tyrosine phosphatase, is able to induce tyrosine phosphorylation on human lymphocytes

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Abstract

CD148 is a new cluster of differentiation defined in the VI International Workshop on Leucocyte Differentiation Antigens. It has been identified as the hematopoietic form of a formerly described membrane protein tyrosine phosphatase called HPTPη/DEP-1. Previous data have demonstrated that this molecule is able to give rise to $[Ca^{2+}]_i$ increase. In the present work we show its capability to induce protein tyrosine phosphorylation in human lymphocytes in spite of its intrinsic protein tyrosine phosphatase activity. The induction of kinase activity suggests the involvement of some protein tyrosine kinase based signaling pathway. The activation of this postulated kinase could be carried out through a direct association or via an adapter molecule.

Keywords: Protein kinases/phosphatases; Signal transduction; Cell surface molecules; T-lymphocytes

1. Introduction

Tyrosine phosphorylation is an essential regulatory mechanism used in signal transduction cascades to control cell proliferation, differentiation and migration [1,2]. Regulation of tyrosine phosphorylation is carried out by the concerted activities of protein tyrosine kinases and protein tyrosine phosphatases. Leukocytes express a wide variety of protein tyrosine phosphatases which include cytosolic phosphatases, such as PTP-1C and PTP-1D, or transmembrane phosphatases. An increasing number of receptor function protein tyrosine phosphatases have been identified [3], CD45 being the most relevant one.

CD148 was defined during the VI International Workshop on Lymphocyte Differentiation Antigens [4] using two monoclonal antibodies: A3 and 143.41, the last one produced and studied in our laboratory. The CD148 molecule is present on different subpopulations of peripheral blood cells with different levels of expression, showing its highest expression on granulocytes, and being present at intermediate intensity on monocytes and lymphocytes. After immunopurifying and partial microsequencing the protein, CD148 was identified as the type III transmembrane protein tyrosine phospahatase HPTPη/DEP-1 (de la Fuente et al. submitted for publication), previously cloned by two independent groups [5,6]. This molecule was shown to have an extracellular portion composed of eight or ten fibronectin III domains, a transmembrane segment and an intracytoplasmic tail which contains a single protein tyrosine phosphatase domain, in contrast with most receptor protein tyrosine phosphatases which contain a tandem repeat of two cytoplasmic phosphatase domains. The presence of fibronectin III motifs in the extracellular domains suggests its possible involvement in cell adhesion processes. On the other hand, the existence of the phosphatase domain in the intracytoplasmic tail evidences its participation in signal transduction.

Previous results in our laboratory have shown that CD148 crosslinking was able to induce Ca^{2+} mobilization (de la Fuente et al. submitted for publication). This

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effect could be abolished by the addition of genistein, a known protein tyrosine kinase inhibitor, as well as by co-crosslinking with CD45, suggesting the involvement of a tyrosine kinase based signaling. In the present paper we have analyzed the ability of CD148 to induce tyrosine phosphorylation and compared it with the effect of CD45, a protein tyrosine phosphatase also present on the membrane of lymphocytes.

2. Material and methods

2.1. Cells

Blood samples were obtained from healthy adult donors. Peripheral blood mononuclear cells were isolated by centrifugation over Ficoll-Hypaque (Pharmacia LKB, Uppsala, Sweden) density-gradient sedimentation.

2.2. Monoclonal antibodies

The following mAbs were produced in our laboratory and ascribed to their CDs through one of the International Workshops on Human Leukocyte Differentiation Antigens: 143–41 (CD148, IgG1), CRIS-7 (CD3, IgG2a) and 72-5D3 (CD45, IgG2a).

2.3. Detection of tyrosine-phosphorylated proteins

Cells (5×10^6) were incubated with the different monoclonal antibodies (10 μg) for 15 min at 4°C followed by the addition of cross-linking rabbit anti-mouse immunoglobulins (5 μg). Incubation was terminated after different periods of time by the addition of 1 ml of ice-cold stop buffer (50 mM Hepes, 150 mM NaCl, 100 mM NaF, 10 mM EDTA, 10 mM $Na_4P_2O_7$, 2 mM Sodium Pervanadate, 2 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml pepstatin, 1 μg/ml leupeptin, 100 μM PAO). Cells were pelleted and lysed with stop buffer containing 1% NP-40. Proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. Phosphotyrosine-containing proteins were probed with anti-phosphotyrosine monoclonal antibody PY-20 from Santa Cruz Biotechnology (Santa Cruz, CA) and horseradish peroxidase conjugated rabbit anti-mouse and visualized by fluorography with enhanced chemiluminiscence (ECL) reagent (Amersham, Buckinghamshire, UK).

3. Results and discussion

In order to confirm the capacity of CD148 to induce protein phosphorylation an assay consisting in a phosphotyrosine immunoblot of differently stimulated cell lysates was developed. As can be observed in Fig. 1, after CD3 crosslinking a neat pattern of protein tyrosine phosphorylation was detected, as has been previously described [7]. In a similar way, after CD148 crosslinking also a clear pattern of phosphorylation could be observed. Likewise, an increase in tyrosine phosphorylation was observed after crosslinking CD45, a known membrane protein tyrosine phosphatase used as a control. In addition to the phosphorylation of some substrates in common with both CD3 and CD45, CD148 was able to induce tyrosine phosphorylation of some specific substrates (e.g. 52 Kd). On the other hand, some substrates clearly hyperphosphorylated after CD3 (e.g. 56 Kd) and CD45 (e.g. 32 Kd) stimulation were not detected after CD148 stimulation.

These results show that the CD148 molecule participates in some signal transduction pathway that involves protein tyrosine phosphorylation even though it has a phosphatase activity. This apparent contradiction could be explained by the possible dephosphorylation of some substrates whose kinase activity would be activated by this dephosphorylation. Actually, the requirement of CD45 in antigen-induced receptor signaling is based on its regulatory capacity on the Src-family protein tyrosine kinases $p56^{lck}$ and $p59^{fyn}$ [8]. By dephosphorylating a negative regulatory tyrosine

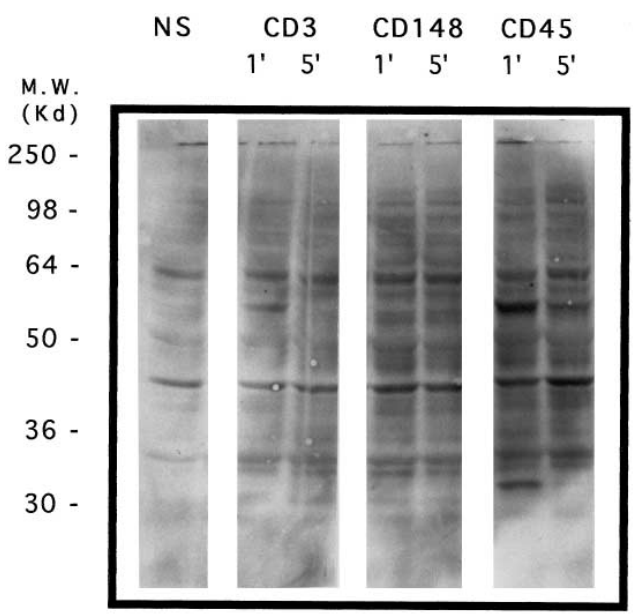

Fig. 1. CD148 crosslinking induces tyrosine phosphorylation on lymphocytes. Cells were left untreated (NS) or incubated with CD3, CD148 or CD45 and after washing, goat anti-mouse was added. The cells were incubated during the indicated times, the reaction was stopped by the addition of stop buffer and cells were lysed with lysis buffer. Total lysates were subjected SDS-PAGE, transferred to nitrocellulose paper and tyrosine phosphorylated proteins were detected by immunoblot with anti-phosphotyrosine antibody.

phosphorylation site at their carboxyl termini, CD45 causes an increase in kinase activity. In fact, it has been shown that $p56^{lck}$ co-immunoprecipitates with CD45 thus establishing a link between these two molecules [9]. Although we have no data on the effect of CD148 on $p56^{lck}$, no clear phosphorylation of substrates around 56 Kd was detected after CD148 stimulation. In any case, the observed increase in tyrosine phosphorylation suggests the implication of some tyrosine kinase which could interact with CD148 directly or through an adapter.

The 3BP2 protein is a possible candidate as an adapter molecule. A potential binding site of the SH2 domain of the 3BP2 protein, a YEN(L/V) motif, has been described by Kuramochi et al. [10] in the cytoplasmic tail of byp, the murine equivalent of human CD148. The presence of this motif is also observed in the cytoplasmic tail of human CD148. The 3BP2 protein contains a proline-rich domain and a pleckstrin-homology domain (PH). The PH domain has been found on several intracellular proteins implicated in signaling [11] and has been postulated to mediate regulated protein–protein interactions during signaling, in a similar manner to the SH2 domain. In addition to CD148 and other type III human phosphatases like HPTP-β and GLEPP-1, the 3BP2 binding domain is found on some other proteins present in the membrane of haematopoietic cells: CD19, CD72, human high affinity epsilon receptor, human EPO receptor and human G-CSFR [12]. A common characteristic of all these molecules is their capability to induce tyrosine phosphorylation. Whether the 3BP2 molecule is implicated on the signal transduction pathway through CD148 or there is a direct interaction with some protein tyrosine kinase remains to be elucidated.

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