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REVIEW

Cybr, CYTIP or CASP: An attempt to pinpoint a molecule's functions and names

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Abstract

Over the last decade several groups, including ourself, have published a series of findings on a molecule expressed in leukocytes. The molecule was termed Cybr, CYTIP or CASP for its functions and PSCDBP for its binding properties. In this review we attempt to chronicle and combine the findings on the molecule to gain an overview of its features.

Keywords: Cybr; CYTIP; CASP; Cytohesin; Dendritic cells

Contents

Dendritic cells are crucial for the regulation of antigen-specific immune responses, which depend on an effective interaction of dendritic cells with T-cells, a process involving adhesion molecules. Dendritic cells are involved in a multitude of innate and adaptive immune mechanisms and shape the outcome of the immune response both regarding the quantity and quality. In the absence of inflammatory and infectious stimuli dendritic cells in the peripheral tissues and in lymphoid organs have the ability to take up and process antigen but are unable to initiate antigen-specific immune responses. For this, dendritic cells undergo phenotypical and anatomical changes, e.g. the expression of surface maturation markers and the migration from peripheral tissues and organs to secondary lymphoid organs. This process is referred to as maturation and depends both

Abbreviations: ARF, ADP-ribosylation factor; CASP, cytohesin-associated scaffolding protein; Cybr, cytohesin binder and regulator; CYTIP, cytohesin interacting protein; EGF, epidermal growth factor; EGFP, enhanced green fluorescent protein; GRASP, GRP-1-associated protein.

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on endogenous and exogenous stimuli from the innate immunity. Depending on the dendritic cell subset and/or these stimuli, dendritic cell maturation can lead to different forms of adaptive immune responses, e.g. immunity vs tolerance, CD4 vs CD8 T-cell response, TH1 vs TH2 vs TH17 immune responses. One major control point of this regulation are the conditions of maturation induction (Enk, 2005; Koebel et al., 2007; Lutz et al., 2006; Steinman, 2007; Trombetta and Mellman, 2005; Villadangos and Schnorrer, 2007). In an attempt to better understand the maturation of dendritic cells on a molecular level we tried to identify proteins induced by pro-inflammatory mediators during *in vitro* maturation of dendritic cells.

History of identification

In approaches designed to isolate molecules induced in dendritc cells during maturation, one protein caught our attention because of its abundant expression in mature dendritic cells, increasing at least 20-fold during maturation. Based on the deduced amino acid sequence, we identified several protein–protein interaction sites, one of which we assigned to cytohesin-1. In 1998 we deposited the cDNA sequence for this protein named cytohesin-binding protein HE in the Genbank with the accession number AF068836. It was similar to the sequence of a protein termed B3-1 with the L06633, submitted by Dixon et al. (1993), but somewhat longer and with a different start codon. On the basis of its deduced amino acid sequence B3-1 was classified as a non-secreted non-membrane-bound protein containing an unusually long leucine zipper, a putative nuclear targeting sequence and a motif found in many oncogenes, transcription factors and interleukins. Its mRNA was found to increase 2-fold in NK/T cells upon stimulation with IL-12 and 25% upon stimulation with PHA/PMA (Dixon et al., 1993). The latter sequence was later assigned to the Chromosome 2 band q11.2 and renamed PSCDBP (Pleckstrin homology Sec7 and coiled coil domains binding protein) (Kim, 1999).

Functional characterization *in vitro*

In vitro functional characterization of the molecule started in 2002 with the finding that it binds to the coiled coil domain of the cytohesin/ARNO family (Boehm et al., 2003; Mansour et al., 2002; Tang et al., 2002). The molecule was now named cytohesin binder and regulator (Cybr, Tang), cytohesin-associated scaffolding protein (CASP, Mansour) and cytohesin interacting protein (CYTIP, Boehm), respectively. From here on we will use the name used by the authors whose data we are referring to. Cybr was isolated from a microarray of IL-12- and IL-2-stimulated NK3.3 cells and PBMC as a more prominently expressed and multiply isolated cDNA. Analyses of the predicted amino acid sequence revealed a PDZ domain and a coiled coil domain and sequence homology to GRP-1-associated protein (GRASP). The expression pattern of Cybr mRNA showed the largest amounts in thymus, spleen, lung, peripheral blood leukocytes, lymph node and bone marrow, as opposed to GRASP, which is reported to be primarily expressed in brain, lung and heart. In addition to the before-mentioned binding of Cybr to cytohesin-1 via the coiled coil domains of both proteins, an additive effect of Cybr on the cytohesin-1-mediated acceleration of guanosine 5′ (O) thiotriphosphate binding by ADP-ribosylation factor (ARF) 1 was shown, implicating a potential link between cytokines and ARFs (Tang et al., 2002). For CASP, in addition to the binding to cytohesins, it was shown that its intracellular localization is perinuclear in transfected COS-1 cells and that it colocalizes with cytohesins to membrane ruffles upon stimulation with epidermal growth factor (EGF), when both molecules are overexpressed in COS-1 cells. The structural similarity of GRASP and CASP and their capability of interacting with multiple members of the cytohesin family suggests a possible function as scaffolding proteins with a role in the ARF-mediated vesicle formation (Mansour et al., 2002). CYTIP was again shown to interact with cytohesin-1, and to be up-regulated during the maturation of monocyte derived dendritic cells, which also express cytohesin-1 but not cytohesin-2. In Jurkat cells transfected with both cytohesin-1 and CYTIP the cytohesin-1-mediated adhesion of LFA 1 to ICAM-1 is inhibited, accompanied by the detachment of the CYTIP-cytohesin-1 complex from the plasma membrane (Boehm et al., 2003). Further studies on CYTIP aimed at understanding the role of the endogenous molecule in human monocyte-derived dendritic cells. CYTIP accumulates at the contact zone between dendritic cells and T-cells in co-cultures. When it is silenced in these cells they keep longer contacts with T-cells and the stimulatory capacity for antigen-specific autologous CD8+ T-cells is diminished, indicating a role for CYTIP in the de-attachment of T-cells from dendritic cells (Hofer et al., 2006). Cybr expression was later found to be induced in primary T-cells by stimulation with PHA and when overexpressed in Jurkat cells to participate in the TCR signalling by regulating Vav phosphorylation and enhancing JNK and p38 MAPK upon CD3 crosslinking. This leads to enhanced AP-1 transcriptional activity and involves co-operation with NFAT · AP-1 (Chen et al., 2006). Finally, an interaction of CASP with sorting nexin 27 via their PDZ domains and their co-localization in early endosomal compartments has recently been reported implicating a function of this interaction in endocytic