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Syndecan-2 and -4 expressed on activated primary human CD4+ lymphocytes can regulate T cell activation

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Abstract

Syndecans bind to cell adhesion molecules, growth factors and cytokines, and can act as coreceptors, and in this way modulate leukocyte cell function. Here, expression of the syndecans on primary human CD4 T cells was examined. Cell stimulation dramatically increased the amount of syndecan-4, and in a lower extent that of syndecan-2. Expression of syndecan-2 and -4 show different induction kinetics. Whereas syndecan-4 expression is fast and significant, that of syndecan-2 is more delayed and short-lived decreasing its mRNA expression at day 4. Both CD45RA+ naive and CD45RA− memory CD4 T cells express syndecan-2 and -4 upon activation. When incubated with human peripheral blood lymphocytes in a mixed leukocyte reaction, anti-syndecan-4 but not anti-syndecan-2 antibodies, decreased T cell proliferation. However, cross-linking of cell-bound syndecan-2 or syndecan-4 via immobilized antibodies blocked proliferation and decreased TNF production of T cells in the presence of optimal levels of anti-CD3. These findings suggest that syndecan-2 and -4 act as inhibitors of T cell activation. We also investigated the role that MAPK signalling pathways play in control of syndecan expression in T cells. We show that production of syndecan-2 but not syndecan-4 requires signaling via p38 MAP kinase α/β in T CD4 cells. As mechanisms that confer syndecan-2 expression are unknown, we analyse the chromatin hypersensitivity of syndecan-2 promoter proximal region in Jurkat T cells and endothelial cells. The analysis reveals a chromatin accessible site in the +3.5 kb intronic region, concomitant with a region showing high evolutionary conservation. We isolate and analyse 5′-flanking regions of human syndecan-2 gene, by transfection assays. The +3.5 kb hypersensitive site in the intronic region demonstrates basal promoter activity in Jurkat. This study provides evidence for the up-regulation of syndecan-2 and -4 in human primary CD4 T cells during in vitro activation and suggest an inhibitory role for these syndecans in CD4 T cells.

Keywords: Heparan sulphate proteoglycans; T-cell proliferation; p38 MAPK; DNAse hypersensitivity

1. Introduction

Syndecans are type I transmembrane heparan sulphate proteoglycans (HSPG) expressed in a developmental and cell type-specific pattern, which participate in diverse biological processes (Bernfield et al., 1999; Rapraeger, 2001; Iozzo and San Antonio, 2001; Couchman, 2003). Via the heparan sulphate chains, syndecans bind to the extracellular matrix as well as to a variety of cytokines, chemokines and growth factors thus modifying their local concentration, stability and accessibility to receptors. Binding to heparan sulphate helps to confine cytokines to lymphoid organs (Wrenshall and Platt, 1999). As cell-surface receptors for chemokines and L-selectin, syndecans play a role in inflammation (Gotte, 2003; Charnaux et al., 2005; Parish, 2006; Alexopoulou et al., 2007). Human T-cell lines may express HSPG (Patel et al., 1993) and they can also be induced in primary T cells (Clasper et al., 1999; Jones et al., 2005), but the type of proteoglycan expressed and its regulation in human T cells is poorly characterized.

Of all the syndecans, syndecan-4 is the most ubiquitously expressed, whereas the other three (syndecan-1, -2 and -3) have a restricted tissue distribution. Mice with a disrupted syndecan-4

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gene are viable, but they show increased mortality due to septic shock (Ishiguro et al., 2001), delayed healing of skin wounds and impaired angiogenesis in the granulation tissue (Echtermeyer et al., 2001). Syndecans in general and syndecan-4 in particular cooperate with integrins during cell-matrix interactions for the assembly of focal adhesions and actin stress fibers (for references see (Morgan et al., 2007)). The cytoplasmic region of syndecan-4 encompasses PDZ-domain- and protein kinase Cα-binding sites and in this way, syndecan-4 can initiate signalling in response to extracellular signals (Grootjans et al., 1997; Koo et al., 2006), including PKCα/Rac1 pathway (Saoncella et al., 2004; Bass et al., 2007). Additional membrane-binding proteins interacting with syndecan-4 cytoplasmic domain are being characterized (Yoo et al., 2005).

Syndecan-2 plays several essential roles during vertebrate development. It can induce dendritic spine formation in hippocampal neurons (Ethell et al., 2001), is required for *zebrafish* vascular development (Chen et al., 2004a) and mediates left–right asymmetry in *xenopus* and *zebrafish* through the actions of TGF-β family members (Kramer et al., 2002; Kramer and Yost, 2002). In the adult, syndecan-2 is expressed in connective tissue but also in myeloid cells (Clasper et al., 1999). The induction of syndecan-2 in human macrophages by inflammatory stimuli regulates fibroblast growth factor-2 action (Clasper et al., 1999).

To date, only the murine syndecan-1 promoter has been characterized (Vihinen et al., 1996; Jaakkola et al., 1997, 1998b): the promoters of the other syndecan family members have not been examined in detail. The transcriptional enhancer of syndecan-1 (FGF-inducible response element: FiRE) is located 10 kb upstream of the transcriptional start site, and is inducible by EGF or FGF-2 in a tissue-specific form (Jaakkola et al., 1997, 1998b). The enhancer FiRE which includes AP-1 elements controlled by the Ras/Erk and PKA pathways (Pursiheimo et al., 2000, 2002), controls syndecan-1 in epithelial cells during wound healing (Jaakkola et al., 1998a; Rautava et al., 2003). Syndecan-4 expression in epithelial cells and macrophages can be induced by TLR agonists in an NFkB-dependent manner (Smith et al., 2006). NFkB also mediates TNF-dependent up-regulation of syndecan-4 in endothelial cells (Zhang et al., 1999b). Little is known about the regulation of syndecan-2 expression (Essner et al., 2006). Moreover, the signalling pathways, promoter elements and transcription factors regulating *syndecan-2* and *-4* genes have not yet been characterized.

In this study, we characterized the expression of syndecan family members by human primary T cells. We demonstrated that upon stimulation, CD4 T cells express syndecan-2 and -4, being the syndecan-2 up-regulation dependent on p38 MAPK activity. It was also a priority to assess the roles of the expressed syndecans in T cell activation. Cross-linking of syndecan-2 and -4 in T cells inhibited their proliferation, suggesting that syndecans can control T cell activation. Finally, to understand the molecular mechanisms involved in the regulation of syndecan-2, we have located different chromatin hypersensitive sites. The functionality of some of these sites has been tested in transfection assays.

2. Materials and methods

2.1. Materials and antibodies

SB203580, PD098059 and SP600125 (all from Tocris, Madrid, Spain) were dissolved in DMSO at 20, 60 or 100 mM, respectively, and stored at −20 °C. Anti-syndecan-2 is a rabbit polyclonal Ab raised against the cytoplasmic tail (Granes et al., 1999). Anti-syndecan-4 mAb was from Santa Cruz Biotechnology (reference sc-12766). Anti-CD3 is a mouse IgG2a mAb (33-2A3 workshop II) and was used purified, or as a hybridoma supernatant at 1:1000 dilution, which was shown to produce maximum TNFα secretion in Jurkat T-cells. Anti-CD28 is a mouse IgM mAb (CK243, kindly donated by Dr. Pedro Romero; Ludwig Institute for Cancer Research, Lausanne Branch, Switzerland). It was used as a hybridoma supernatant at 1:12 dilution, which had previously been optimized by titration to produce maximum TNFα secretion in combination with anti-CD3 in Jurkat T cells. Anti-CD4 is a mouse IgG_{2A} mAb (72-5A4 workshop II). Anti-CD45RA is a mouse IgG1 mAb (111-1C5 workshop III).

2.2. Monoclonal antibody production

mAb reactive with syndecan-2 antigen were generated by the fusion of NS-1 myeloma cells with splenocytes from mice immunized three times with 300.19 cells transfected with human *syndecan-2*. Three hybridoma-producing antibodies against syndecan-2 were selected and subcloned by limiting dilution. Ab isotypes were determined using a mouse mAb isotyping kit (Roche Applied Science, Sant Cugat, Spain). mAbs were purified using the Affi-Gel Protein A MAPS II kit (Bio-Rad Barcelona, Spain) from concentrated supernatants obtained from hybridoma cultures in INTEGRA CL 1000 flasks (Integra Biosciences, Cultek, Madrid, Spain). The anti-syndecan-2 mAb used in this study is an IgG1 (clone sdc2.1.186).

2.3. Preparation of human T lymphocytes

Primary human T cells were obtained from buffy coats from blood donors at the Hospital Clínic and Hospital Vall d'Hebron (both in Barcelona). Batches of blood (25 mL) were layered on top of 20 mL Ficoll 1.007 density gradient (Lymphoprep ref. 1114545, Axis-Shield, Reactiva, Barcelona, Spain) and centrifuged at $400 \times g$ for 20 min at room temperature. After centrifugation, the peripheral blood mononuclear cell layer was collected and washed twice in DMEM and the cells were counted. Cells were resuspended at $10–12 \times 10^6$ cells/mL in DMEM supplemented with 10% heat-inactivated foetal calf serum (FCS) and distributed in 75 cm^2 plastic flasks, 150×10^6 cells per flask (Becton Dickinson, Madrid, Spain). After 20–30 min at 37 °C, the supernatants were collected and the plastic-adhered monocytes discarded. Peripheral blood lymphocytes (PBL) in supernatants were resuspended at 10^6 cells/mL in DMEM-0.5% FCS, penicillin and streptomycin, and they were cultured overnight before stimulation. The primary T lymphocytes in