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Cathepsin A is expressed in primary human antigen-presenting cells

Michael Reich[a], Klaus-Dieter Spindler[b], Michael Burret[b], Hubert Kalbacher[c], Bernhard O. Boehm[a], Timo Burster[a,*]

[a] Division of Endocrinology and Diabetes, Center for Internal Medicine, University Medical Center Ulm, Ulm, Albert-Einstein-Allee 23, 89081 Ulm, Germany
[b] Insitute of General Zoology and Endokrinology, Ulm, Germany
[c] Medical and Natural Sciences Research Centre, University of Tübingen, Germany

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ABSTRACT

Cathepsins are expressed in antigen-presenting cells (APC). These cathepsins are known to regulate antigen processing and degradation of the invariant chain (Ii) into the class II-associated Ii peptide (CLIP), which occupies the peptide-binding groove of the major histocompatibility complex (MHC) class II molecule. Previous studies have identified the serine carboxypeptidase cathepsin A (CatA) in various tissues and cells; however, it is not clear whether CatA is also expressed in primary human APC. We demonstrate the expression of CatA in B lymphoblastoid cells (BLC), primary human B cells, both subsets of myeloid dendritic cells (mDC1 and mDC2), as well as in plasmacytoid DC. PMSF or lactacystin-mediated inhibition of serine proteases in BLC-derived lysosomal proteases resulted in the inhibition of amino acid release from the C-terminal end of two model peptides. This inhibition did not occur by using a proline rich peptide. Our data suggest that CatA is involved in the C-terminal fine-tuning of antigenic T cell epitopes in human APC.

1. Introduction

Proteolysis of antigens and the invariant chain (Ii) in antigen-presenting cells (APC) is required for the generation and presentation of antigenic peptides to $CD4^+$ T cells *via* the major histocompatibility complex (MHC) class II molecules. Engagement of the T cell receptor with the peptide/MHC II complex can trigger signaling pathways that promote immunity, as well as tolerance. This engagement is dependent on the presence of inflammatory signals and the differentiation states of the APC and the T cell (summarized in [1]).

Antigen processing in the MHC class II endocytic compartments is critical for the formation of potent T cell epitopes. Several classes of proteases, including serine, cysteine, and aspartyl, are necessary to generate these epitopes. The serine protease, cathepsin G (CatG) [2–4], the cysteine proteases (e.g. CatB, C, F, H, L, S, V, X, and asparagine endopeptidase) [5–11], and the aspartyl proteases (e.g. CatD, and E) [12–15] are active enzymes of the endocytic compartments. These proteases function to process antigens until they can bind to MHC class II molecules.

The serine protease CatA, a member of the serine S10 family of proteases, has been identified as the major lysosomal protease in the rat kidney [16]. The pro-form of CatA is assembled by the catalytically active 32 kDa subunit along with the 20 kDa polypeptide resulting in a 54 kDa monomer [17]. A high molecular weight CatA complex is formed by the binding of two CatA monomers, which then traffics to the lysosomal compartment. CatA exhibits a pH range between 4.6 and 5.8, and functions by releasing single amino acids from the C-terminal end of a peptide. In general, CatA appears to prefer the release of hydrophobic amino acid residues [18,19]. In addition to its carboxypeptidase activity, CatA also acts as a deamidase and esterase [20]. Studies have shown that CatA is able to regulate blood pressure in both rats [18] and mice [21] through the degradation of angiotensin or endothelin-1. Additionally, CatA has also been implicated in the process of autophagy, which occurs after the digestion of lysosome-associated membrane protein type 2a (lamp2a) [22]. Furthermore, defects in the enzymatic activity of CatA have been reported to cause galactosialidosis, a lysosomal storage disorder in humans [23,24]. CatA is mainly expressed in platelets [20], lymphokine-activated killer cells (LAK) [25], spleen cells, fibroblasts, kidney, liver [26], melanoma cells [27], peripheral blood mononuclear cells (PBMC) [28] and human alveolar macrophages [29]. However, CatA

Abbreviations: APC, antigen-presenting cells; BLC, B lymphoblastoid cells; Cat, cathepsin; mDC, myeloid dendritic cells; MBP, myelin basic protein; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cells; PMSF, phenylmethane sulfonyl fluoride; pDC, plasmacytoid DC; PMN, polymorphonuclear leukocytes.

* Corresponding author. Tel.: +49 731 500 44521; fax: +49 731 500 44519.
E-mail address: tburster@stanford.edu (T. Burster).

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has not yet been described in primary B cells or dendritic cells (DC).

This report describes CatA in B lymphoblastoid cells (BLC), primary human B cells, as well as both subsets of myeloid dendritic cells (mDC1 and mDC2). Our studies have also identified CatA in plasmacytoid DC (pDC). Furthermore, the incubation of CatA, or BLC-derived lysosomal cathepsins, with two model antigenic peptides resulted in the degradation of C-terminal hydrophobic amino acids from each of the peptides. However CatA was unable to degrade a proline-rich peptide.

2. Materials and methods

2.1. Cells

Human peripheral blood mononuclear cells (PBMC) were isolated from the buffy coats of healthy HCMV-seronegative blood donors by density gradient centrifugation. B cells ($CD19^+$), myeloid dendritic cells (mDC1, $CD1c^+$ or mDC2, $CD141^+$), plasmacytoid DC (pDC, $CD303^+$), T cells ($CD4^+$ and $CD8^+$), $CD14^+$, and polymorphonuclear leukocytes (PMN) were positively selected using the appropriate magnetic cell separation kit (Miltenyi Biotec, Bergisch Gladbach, Germany), following the manufacturer's protocol. The use of human cells for *in vitro* studies was in accordance with IRB regulations.

2.2. Immunoblot

Cells were lysed (10 mM Tris [pH 7.5], 150 mM NaCl, and 0.5% NP-40) and adjusted for equal total protein (quantified by the Bradford Assay). 20 μg of cell lysate were resolved on a 12% SDS-PAGE gel and 50 ng of recombinant CatA (R&D Systems, Wiesbaden, Germany) was used. Levels of CatA protein were detected by using a goat anti-human CatA antibody (R&D Systems) followed by a secondary HRP-conjugated antibody (Sigma-Aldrich, Steinheim, Germany). Anti-beta-actin (Sigma–Aldrich) was used as a loading control.

2.3. In vitro processing of peptides

VVHPFPPIVTPPPP (MBPMu4, [30]), FVNQHLCGSHLV (DCins8), and FVNQHLCGSHLVEAL (DCins10) peptides were synthesized by the solid phase Fmoc strategy on the multiple peptide synthesizer Syro II (MultiSynTech, Witten, Germany). Activation and coupling were performed with TBTU, HOBT, and NMM (Merck, Darmstadt, Germany). Peptides were purified by reversed-phase HPLC using a C18 column 125 × 8 (Grom, Herrenberg, Germany) and analyzed by mass spectrometry (Reflex IV, Bruker Daltonics, Bremen, Germany). Lysosomal proteases from BLC were generated by differential centrifugation and characterized, as previously published [31]. Peptides (0.2 μg/μl) were incubated with either recombinant human CatA (2 ng/μl, R&D Systems) or with 0.05 μg/μl of BLC-lysosomal proteases (B cell line cox) pre-incubated with both pepstatin A (30 μM) and E64 (30 μM, Calbiochem) for 15 min. As a control, BLC-lysosomal proteases were additionally pre-incubated with phenylmethane sulfonyl fluoride (PMSF, 5 mM, Sigma–Aldrich) or lactacystin (30 μM, Enzo Life Sciences, Lörrach, Germany) prior to the addition of the peptides. The digest was performed for 2 h or 24 h at 37 °C in reaction buffer (0.1 M citrate pH 5.0, 2.5 mM DDT). The digestion pattern was resolved by reversed-phase HPLC using a C8 column 125 × 2 (Grom) and the fragments were analyzed by mass spectrometry (MALDI-TOF, Reflex IV, Bruker Daltonics) and the ExPASy Proteomics Server (Swiss Institute of Bioinformatics, Lausanne, Switzerland).

2.4. Immunohistochemistry

B cells and mDC1 were seeded onto poly-D-lysine coated 8-well culture slides and allowed to adhere for 1 h at 37 °C. Cells were washed with PBS and fixed in ice-cold methanol/acetone (1:1, v/v) at −20 °C for 10 min. After additional wash steps with PBS, the samples were blocked with human inactivated AB serum (10% in PBS) for 10 min. The primary antibody, goat anti-human CatA (1:100, R & D Systems), was added for 1 h, followed by several washes with PBS. The cells were then blocked with 10% inactivated human AB serum for 5 min and incubated with the secondary antibody, rabbit anti-goat Cy3 (1:200, Sigma–Aldrich), followed by extensive washing with PBS. Photographs were taken using a fluorescence microscope (Zeiss, Jena, Germany). Original magnification: 40×.

3. Results

3.1. Localization of CatA in primary human antigen-presenting cells

We first examined the expression of CatA in peripheral blood mononuclear cells (PBMC). Crude cell lysate from PBMC were loaded onto an SDS-PAGE gel and analyzed by immunoblotting with a CatA-specific antibody. As shown in Fig. 1A, CatA was detected in PBMC as the pro-form (∼50 kDa) and the active-form (∼32 kDa). Based on these results, we next sought to characterize the expression of CatA in different human cells. Therefore, B cells ($CD19^+$), two subsets of myeloid DC (mDC1 and mDC2), plasmacytoid DC (pDC), T cells ($CD4^+$ and $CD8^+$), monocytes ($CD14^+$), and polymorphonuclear leukocytes (PMN) from PBMC were isolated by using magnetic bead techniques. Thereafter, CatA expression was detected by a CatA-specific immunoblot. We found comparable levels of CatA protein in mDC1, mDC2, and pDC, and lower levels of expressed CatA in B cells (Fig. 1B). Of note, recombinant CatA (rCatA) is histidine-tagged and therefore migrates differently compared to PBMC-derived CatA. The analysis of CatA in further cells will be found in the supplementary data section.

Moreover, the intracellular location of the CatA protein was determined B cells and mDC by immunohistochemistry with a CatA specific antibody. CatA was detected in all of the analyzed cells (Fig. 1C). These results clearly demonstrate the expression of CatA in major antigen-presenting cells.

3.2. CatA resolves hydrophobic amino acids, but not proline residues from the C-terminal end of selected peptides

In order to determine whether the use of certain peptides might function as substrates for CatA, two peptides with a hydrophobic C-terminal end (DCins8 and DCins10) and one proline rich peptide (MBPMu4) were incubated with recombinant human CatA. As demonstrated in Fig. 2A, CatA releases single amino acid residues from the C-terminal end of DCins 8 and DCins10, but not from MBPMu4. Furthermore, the activity of CatA was also shown to be inhibited by 30 μM lactacystin (data not shown). To further investigate the possibility that CatA is also active in APC-derived lysosomes, we dissected B lymphoblastoid cells (BLC)-derived lysosomal cathepsins by using a CatA-specific immunoblot. As shown in Fig. 2B, expression of the CatA protein was verified in both the cell lysate and lysosomes from BLC. Therefore, CatA is present in lysosomes of BLC and consequently BLC-derived lysosomes are capable of peptide digestion. BLC-derived lysosomal cathepsins were pre-incubated with the aspartyl protease inhibitor, pepstatin A, and the cysteine protease inhibitor, E64, in the presence or absence of PMSF or lactacystin. The peptide substrates MBPMu4 and DCins10 were then added to each of the reactions. The MBPMu4