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Cathepsin S inhibition suppresses autoimmune-triggered inflammatory responses in macrophages

Sophia Thanei [a,*], Michel Theron [b], Ana Patricia Silva [b], Bernhard Reis [b], Leonore Branco [a,c], Lucia Schirmbeck [a,c], Fabrice A. Kolb [d], Wolfgang Haap [e], Thomas Schindler [b], Marten Trendelenburg [a,c]

[a] Laboratory of Clinical Immunology, Department of Biomedicine, University Hospital Basel, University of Basel, Hebelstrasse 20, 4031 Basel, Switzerland
[b] Roche Pharma Research and Early Development, Pharmaceutical Sciences, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., Grenzacherstrasse 124, 4070 Basel, Switzerland
[c] Division of Internal Medicine, University Hospital Basel, University of Basel, Petersgraben 4, 4031 Basel, Switzerland
[d] Roche Pharma Research and Early Development, Immunology, Inflammation and Infectious Diseases Discovery and Therapeutic Area, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., Grenzacherstrasse 124, 4070 Basel, Switzerland
[e] Roche Pharma Research and Early Development, Medicinal Chemistry, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., Grenzacherstrasse 124, 4070 Basel, Switzerland

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ABSTRACT

In several types of antigen-presenting cells (APCs), Cathepsin S (CatS) plays a crucial role in the regulation of MHC class II surface expression and consequently influences antigen (Ag) presentation of APCs to $CD4^+$ T cells. During the assembly of MHC class II-Ag peptide complexes, CatS cleaves the invariant chain p10 (Lip10) – a fragment of the MHC class II-associated invariant chain peptide. In this report, we used a selective, high-affinity CatS inhibitor to suppress the proteolytic activity of CatS in lymphoid and myeloid cells. CatS inhibition resulted in a concentration-dependent Lip10 accumulation in B cells from both healthy donors and patients with systemic lupus erythematosus (SLE). Furthermore, CatS inhibition led to a decreased MHC class II expression on B cells, monocytes, and proinflammatory macrophages. In SLE patient-derived peripheral blood mononuclear cells, CatS inhibition led to a suppressed secretion of IL-6, TNFα, and IL-10. In a second step, we tested the effect of CatS inhibition on macrophages being exposed to patient-derived autoantibodies against C1q (anti-C1q) that are known to be associated with severe lupus nephritis. As shown previously, those SLE patient-derived high-affinity anti-C1q bound to immobilized C1q induce a proinflammatory phenotype in macrophages. Using this human *in vitro* model of autoimmunity, we found that CatS inhibition reduces the inflammatory responses of macrophages as demonstrated by a decreased secretion of proinflammatory cytokines, the downregulation of MHC class II and CD80.

In summary, we can show that the used CatS inhibitor is able to block Lip10 degradation in healthy donor- and SLE patient-derived B cells and inhibits the induction of proinflammatory macrophages. Thus, CatS inhibition seems to be a promising future treatment of SLE.

1. Introduction

Cathepsin S (CatS) is a cysteine protease of the papain family and plays an important role in antigen (Ag) processing and presentation as well as in matrix degradation [1]. CatS is present in the lysosomal/endosomal compartments of professional antigen-presenting cells (APCs), namely B cells, macrophages, and dendritic cells (DCs) [2]. In these cells, CatS plays an essential role in the assembly of major histocompatibility complex class II (MHC class II)-Ag complexes and thus facilitates Ag presentation to $CD4^+$ T cells [3–5]. Extracellular Ags are taken up by APCs and are mostly degraded in the lysosomal/endosomal compartments of these cells [6]. The peptides resulting form the degraded Ags are then displayed on the cell surface after the formation of MHC class II-Ag peptide complexes. The intracellular molecular mechanism result-

Abbreviations: Ag(s), antigen(s); Anti-C1q, anti-C1q autoantibodies; APCs, antigen-presenting cells; CatL, Cathepsin L; CatS, Cathepsin S; CLIP, class II-associated invariant chain peptide; DCs, dendritic cells; $DMEM^*$, DMEM supplemented with 1% penicillin/streptomycin; HSA-DMEM, $DMEM^+$ supplemented with HSA; DMSO, dimethyl sulfoxide; FcγR(s), Fcgamma receptor(s); IC_{50}, half maximal inhibitory concentration value; Ig(s), immunoglobulin(s); imC1q, immobilized C1q; imC1q+SLEIgG, anti-C1q bound to imC1q; HMDMs, human monocyte-derived macrophages; HSA, human serum albumin; Ii,, invariant chain; Lip10, invariant chain p10; LPS, lipopolysaccharide; M1, proinflammatory macrophages; M2, anti-inflammatory macrophages; MCP-1, monocyte chemoattractant protein-1; MFI, mean fluorescence intensity; MHC class II, major histocompatibility complex class II; MØ, untreated macrophages; ns, not significant; PBMCs, peripheral blood mononuclear cells; PBS, phosphate buffered saline; PI, Propidium iodide; SLE, systemic lupus erythematosus; SLEIgG, SLE patient-derived IgG; SN, supernatants; TNFα, tumor necrosis factor alpha.

* Corresponding author.
E-mail address: sophia.thanei@unibas.ch (S. Thanei).

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ing in the formation of MHC class II-Ag peptide complexes begins with the assembly of the α- and β-chains of MHC class II in the endoplasmic reticulum with the assistance of the MHC class II Ii chaperone molecule, the invariant chain (Ii). This complex is then transported to the lysosomal/endosomal compartments, where Ii is degraded in a stepwise fashion by different proteases allowing the Ag-binding site of MHC class II to be exposed. In B cells, CatS is the only enzyme that cleaves the invariant chain p10 (Lip10), which is a 10kDa fragment of the MHC class II-associated invariant chain peptide (CLIP), during the assembly of MHC class II-Ag peptide complexes in the lysosomal/endosomal compartments [4,7–9]. In contrast, in murine macrophages, both, CatS and Cathepsin L (CatL), are involved in Ii degradation and Ag processing. Therefore, CatS deficiency does not severely impair Ag processing and presentation in murine macrophages [10]. However, in interferon γ-stimulated murine macrophages CatL does not play a significant role in the degradation of the Ii [11]. These data indicate, that dependent on the stimulus inducing an immune response, MHC class II-dependent Ag presentation by APCs is mainly regulated by CatS.

In addition, CatS has been implicated in pathological processes being associated with atherosclerosis, abdominal aortic aneurysm, and autoimmune diseases including systemic lupus erythematosus (SLE) [12,13]. As a consequence, CatS inhibitors have been developed and are being tested as drug candidates for the treatment of patients suffering from cardiovascular and autoimmune diseases [14,15]. With regard to SLE, Rupanagudi et al. reported that the inhibition of CatS has a therapeutic effect in lupus-prone MRL-Fas(lpr) mice. MRL-Fas(lpr) mice treated with a CatS inhibitor (RO5461111) had significantly lower plasma levels of IL-10 and tumor necrosis factor alpha (TNFα), a reduced number of $CD4^+$ T cells in the spleen, less immunoglobulin G (IgG) deposition in the kidneys, and lower titers of circulating anti-double stranded DNA autoantibodies as compared to untreated mice. Thus, the treatment with the CatS inhibitor improved lupus nephritis, most likely by inhibiting Ag presentation [13].

SLE is a chronic autoimmune disease characterized by B cell hyperactivity, the production of a variety of autoantibodies targeting intra-cellular components and plasma proteins, the formation of immune complexes, and ongoing complement activation and deposition resulting in inflammation and hypocomplementemia [16,17].

Homozygous deficiency of the complement component C1q, the initiator molecule of the classical pathway, is the strongest known disease susceptibility factor for the development of SLE in humans, underlining the important role of C1q in the pathogenesis of SLE [18,19]. However, most SLE patients do not suffer from primary C1q deficiency, but aberrant complement activation is accounted for secondary hypocomplementemia. Low or undetectable C1q levels are frequently observed in SLE patients and are often associated with autoantibodies directed against C1q (anti-C1q). Anti-C1q are present in 20–50% of unselected SLE patients and their occurrence strongly correlates with both low complement levels and severe lupus nephritis [20–22]. Even though, these autoantibodies are linked to renal involvement, the direct evidence how these autoantibodies contribute to the pathogenesis of lupus nephritis is still lacking. Animal models suggested that renal inflammation is only induced by anti-C1q in combination with preformed glomerular C1q-containing immune complexes, requiring both complement activation and Fcgamma receptor (FcγR) triggering [23]. Indeed, more recent studies could confirm that anti-C1q activate the complement system and induce a proinflammatory cytokine response in macrophages by cross-linking of FcγRII [24,25].

It is believed that the complement protein C1q is predominantly produced by myeloid cells [26–30]. In the circulation, C1q is mostly associated with its proteases C1s and C1r to form the C1 complex, the initiator molecule of the classical pathway of complement [31,32]. However, beyond complement activation, C1q has an important role in regulating inflammatory processes, including autoimmunity, and in mediating the clearance of apoptotic cell material [33]. Different reports demonstrated that upon binding of C1q to apoptotic cells [34,35], C1q facilitates the engulfment and clearance of apoptotic cells [36–38], thereby limiting inflammation and autoimmunity during the phagocytosis of dead cell material.

The aims of this study were to compare the accumulation of Lip10 in $CD20^+$ B cells obtained from healthy donors and SLE patients, and to analyze whether the inhibition of CatS might suppress an autoantibody-mediated induction of proinflammatory human monocyte-derived macrophages (HMDMs). For this, CatS inhibition was tested in an *in vitro* model of human proinflammatory (M1) macrophages stimulated by autoimmune, patient-derived anti-C1q.

2. Patients and methods

2.1. Patients

All SLE patients investigated in this study fulfilled at least 4/11 criteria of the American College of Rheumatology [39,40]. Collection and use of serum samples from SLE patients were approved by the local Ethics Committee. All patients had given written informed consent prior to inclusion in the study.

2.1.1. Anti-C1q source

A cohort of 15 SLE patients (Table 1) (EKZ-No.: 110/04; 130/05) was investigated. IgG anti-C1q levels in SLE patients were measured and the optical density values standardized and expressed as arbitrary units (AU) as described previously [25].

2.1.2. Cells

15 ml of heparinized venous blood was obtained from SLE patients (Table 2) (EKZ-No.: 2014/125). Peripheral blood mononuclear cells (PBMCs) and $CD14^+$ monocytes were isolated as described in Section 2.3. However, adherent SLE patient-derived $CD14^+$ monocytes were differentiated into macrophages in DMEM+ supplemented with 10% autologous serum for 7 days. For the analysis of LPS-induced cytokine release, the patient's differentiated macrophages were stimulated as described in Section 2.4.

2.2. Lip10 accumulation assay

2.2.1. Lip10 accumulation in $CD20^+$ B cells obtained from SLE patients and healthy donors

15 ml of heparinized venous blood was obtained from SLE patients (Table 2) or healthy donors. PBMCs were isolated by Ficoll-Paque (Histopaque-1077 (Sigma-Aldrich, St. Louis, MO, USA)) density gradient centrifugation and diluted at 4×10^6 cells/ml in RPMI 1640 medium supplemented with 1% penicillin/strep-

Table 1
Characterization of SLE patients used as a source for anti-C1q.[a]

Sex (females/males)	11/4
Age (years; median (range))	35 (19–68)
Hypocomplementemia (low C3 and/or C4) (yes/no)	11/4
Lupus nephritis (yes/no)	11/4
Positive for anti-C1q	12
Negative for anti-C1q	3

[a] Information at time point of blood sampling.

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