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Opinion

Putative regulation of macrophage-mediated inflammation by catestatin

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Catestatin (CST) is a bioactive cleavage product of the neuroendocrine prohormone chromogranin A (CgA). Recent findings show that CST can exert anti-inflammatory and antiadrenergic effects by suppressing the inflammatory actions of mammalian macrophages. However, recent findings also suggest that macrophages themselves are major CST producers. Here, we hypothesize that macrophages produce CST in an inflammation-dependent manner and thereby might self-regulate inflammation in an autocrine fashion. CST is associated with pathological conditions hallmarked by chronic inflammation, including autoimmune, cardiovascular, and metabolic disorders. Since intraperitoneal injection of CST in mouse models of diabetes and inflammatory bowel disease has been reported to be beneficial for mitigating disease, we posit that CST should be further investigated as a candidate target for treating certain inflammatory diseases.

Highlights

Catestatin (CST), a 21-amino acid peptide derived from proteolytic cleavage of the prohormone chromogranin A, has been reported to have anti-inflammatory and antiadrenergic functions in mice.

CST is thought to be mainly co-released with catecholamines by neuroendocrine cells.

Recent studies suggest that macrophages are also a source of CST and that the anti-inflammatory effects of CST might be attributable to CST produced by macrophages, at least in mice.

We propose that CST produced by macrophages might suppress neuronal and neuroendocrine activity, in an inflammation-dependent manner.

The anti-inflammatory and antiadrenergic peptide catestatin

CgA (see Glossary) is produced and secreted in vertebrates by **neuroendocrine**, **endocrine**, and **enteroendocrine cells**, such as chromaffin cells, pituitary gland, and enteroendocrine cells in the gut [1–6]. Proteolytic conversion of CgA, either before or after its release, gives rise to CST (human $CgA_{352–372}$) [7–10]. The physiological effects of the 21-amino acid peptide CST are well established. It is pleiotropic and functions as a neuroregulatory, antimicrobial, and chemotactic peptide [7,8,11–13] (see Clinician's corner). CST also exerts antiadrenergic effects, as explained below, and has well-known metabolic effects [7,8]. For example, intraperitoneal injection of CST improved glucose and insulin tolerance in diet-induced obese (DIO) mice [14] and resulted in increased expression of genes involved in fatty acid oxidation, decreased plasma concentrations of triglycerides, and reduced fat depot sizes relative to control mice [15]. As further discussed, CST can also have anti-inflammatory effects; based on these findings, it has been hypothesized that, by producing CST, neuroendocrine cells can not only regulate their own activation by negative-feedback inhibition, but also suppress macrophage-driven inflammation in a **paracrine** fashion [7].

However, in this opinion, we argue that CST might also signal in the reverse direction: from macrophages to neuroendocrine cells. First, we discuss the role of CST in suppressing **catecholamine** release and macrophage-driven inflammation. Second, we discuss new evidence suggesting that macrophages are one of the major producers of CST. Third, we explore the contribution of this macrophage-produced CST to the regulation of neuroendocrine and immune systems. Thus, we touch on the potential clinical implications of the putative role of CST in the pathogenesis and progression of certain chronic inflammatory diseases.

CST can suppress catecholamine release from neuroendocrine cells

CST was first described as a **neuropeptide** [16] present, for example, in the ocular retina and nerves of rats [17] and in the human auditory system [18]. Neuroendocrine cells and **adrenergic**

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neurons, co-release catecholamines together with CST [16,19,20]. Neurons and neuroendocrine cells not only produce CST, but CST also affects their activity. For example, microinjection of CST in the rat brain (rostral and caudal ventrolateral medulla) decreased sympathetic nerve activity relative to controls, as measured by electrophysiology, heart rate, and neuronal responses to changes in blood pressure (phenylephrine injection) and hypoxia (in the pressor area of the medulla) [21]. Moreover, intrathecal administration of CST in rats decreased nicotine-induced increases in arterial pressure and splanchnic sympathetic nerve activity compared with untreated animals [22]. In CgA-knockout (KO) mice (CgA-KO; *Chga*$^{-/-}$), intraperitoneal injection with CST normalized the heightened plasma concentrations of the catecholamines **norepinephrine** and **epinephrine**, diminished immobilization-induced increments in blood pressure and heart rate, normalized the diminished baroreflex sensitivity in response to phenylephrine or sodium nitroprusside [23], and restored the heart rate variability of mice, as measured by electrocardiography [24]. Moreover, supporting the observation that CST can reduce catecholamine secretion, CST concentrations increased in circulation, whereas the amount of norepinephrine decreased 36 h after acute myocardial infarction in patients relative to healthy individuals [25].

To study the effects of CST further, a mouse was generated with selective deletion of 63 base pairs in the exon VII region of *Chga* encoding CST (mCgA$_{364–384}$) on a C57BL/6 background [*Chga*$^{del(1284–1346)/del(1284–1346)}$; i.e., CST-KO mice] [14,26]. Relative to wild-type mice, CST-KO mice are hypertensive and exhibit elevated amounts of norepinephrine and epinephrine in the plasma and adrenal gland [26]. These phenotypes are fully reversible with intraperitoneal injection of CST [26].

CST is able to directly suppress catecholamine release by neuroendocrine cells; indeed, experiments with radio-labeled norepinephrine and quantitative electron microscopy showed that CST inhibited catecholamine release in the *in vitro* cultured neuroendocrine rat cell line PC12 [16,19], in primary cultured bovine chromaffin cells [16], and in primary cultures of rat hippocampal neurons [27]. Also *in vivo,* murine experiments showed that intraperitoneal injection of CST could reduce the plasma concentration of nicotine-induced norepinephrine and epinephrine [28]. Moreover, nicotinic stimulation increased *Chga* expression in PC12 cells [29] and in mice expressing the luciferase reporter gene under the control of the *Chga* promoter [28]. Based on these findings, in the neuroendocrine system, CST has been considered to be an **autocrine** neuromodulatory factor enabling neuroendocrine cells to self-suppress their catecholamine release in an activity-based fashion [7].

CST can suppress macrophage-mediated inflammation

CST can also suppress inflammation (Figure 1). For example, relative to wild-type mice, CST-KO mice display elevated concentrations of proinflammatory cytokines, such as tumor necrosis factor α (TNF-α), interferon γ (IFN-γ), C-X-C motif chemokine ligand 1 (CXCL1), C–C motif chemokine ligand 2 (CCL2), and CCL3, in the circulation and heart, which were all reversed by intraperitoneal injection of CST [26]. Moreover, oral feeding of fluorescently labeled dextran showed that CST-KO mice exhibit impaired intestinal barrier function, a hallmark of intestinal inflammation; in addition, elevated amounts of dextran were detected in the blood, which was reversed by intraperitoneal CST injection [30]. Intestinal inflammation was confirmed by elevated colon concentrations of TNF-α, IFN-γ, CXCL1, and CCL2 in CST-KO mice [30].

Several lines of evidence suggest that the anti-inflammatory effects of CST occur primarily by suppressing the infiltration of monocytes (monocyte-derived macrophage precursors) into tissues, as well as by suppressing macrophage-driven inflammation. In accordance with the results mentioned above, CST-KO mice also exhibit increased macrophage infiltration in the

Glossary

Adrenergic neurons: secrete norepinephrine and innervate smooth muscle, cardiac muscle and visceral glands, among others; found in the central and autonomic nervous system; responsible for a variety of basic physiological functions.
Autocrine signaling: cell secretes a messenger (such as a cytokine or catestatin) that binds to the cognate receptors on that same cell.
Catecholamine: group of structurally related neurotransmitters, which includes dopamine, epinephrine (adrenaline), and norepinephrine (noradrenaline).
Chromogranin A (CgA): 49-kDa prohormone secreted by endocrine cells; its proteolytic processing gives rise to several bioactive peptide fragments, including CST.
Endocrine cell: secretes hormones, such as CgA, in the blood in response to stimuli.
Enteroendocrine cell: located in the gastrointestinal tract; produces hormones, such as CgA and serotonin.
Epinephrine (adrenaline): functions as both a hormone and a neurotransmitter. As a hormone, it is produced in the adrenal medulla and neuroendocrine cells, whereas, as a neurotransmitter it is produced by neurons or neuroendocrine cells. Following release, it can activate the sympathetic nervous system by binding to α- and β-adrenergic receptors. Its effects depend on the specific tissue and mostly affect cardiac output or blood pressure, vasoconstriction via smooth muscle contraction and glucose production by glycogenolysis and gluconeogenesis.
Neuroendocrine cell: connects nervous and endocrine systems; can receive signals from neurons via neurotransmitters and, as a result, secrete hormones, such as CgA, and/or neurotransmitters, including catecholamines.
Neuropeptide: small peptides produced by neurons and neuroendocrine cells that act via various G-protein-coupled receptors to modulate cell and synaptic activity.
Norepinephrine (noradrenaline): functions as both a hormone and a neurotransmitter. As a hormone, it is produced in the adrenal medulla, whereas, as a neurotransmitter, it is produced by nerves or neuroendocrine cells. Following release, it can activate

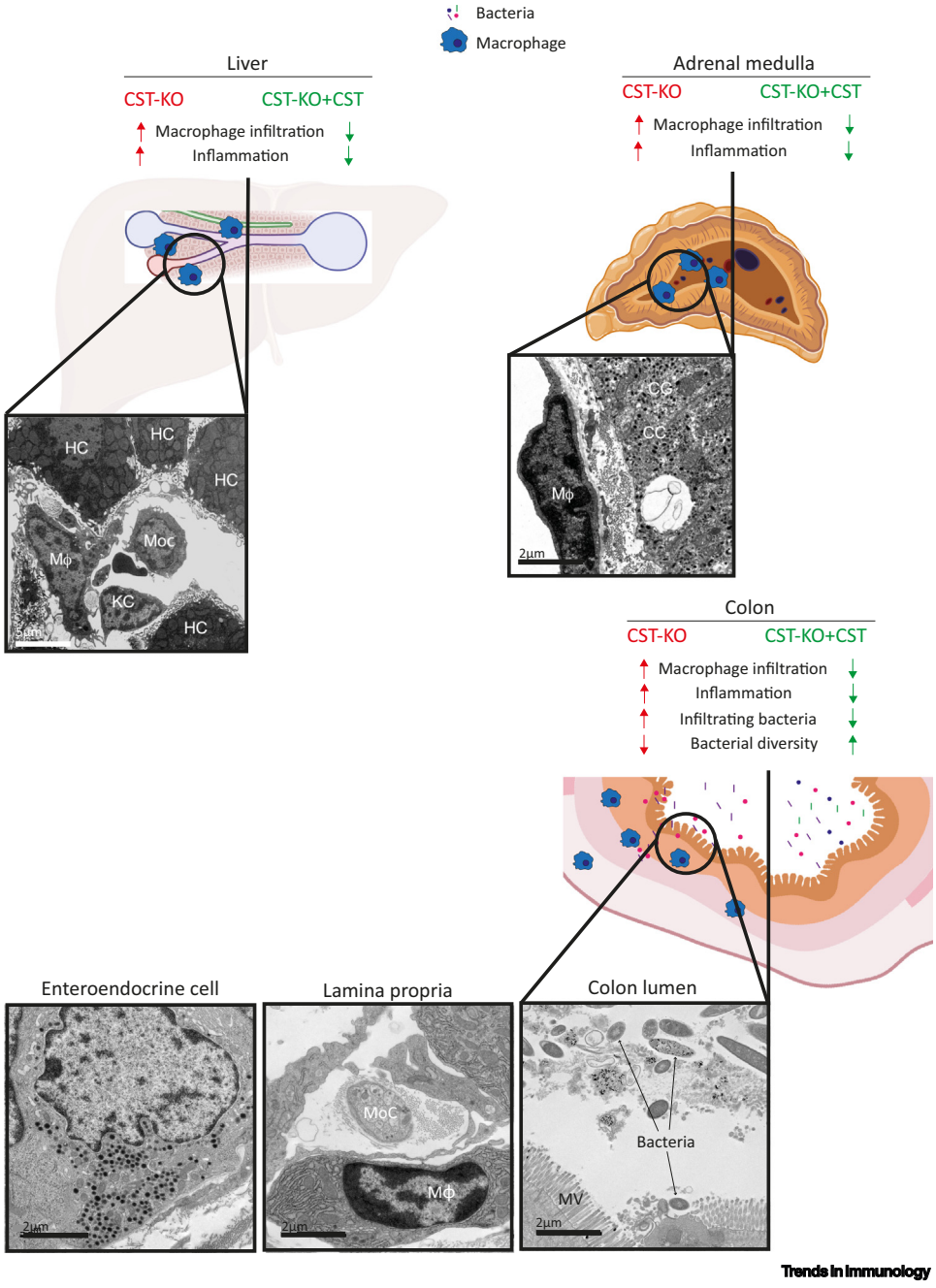

Figure 1. Examples of catestatin (CST) expression in several mouse tissues. For illustrative purposes, representative transmission electron microscopy images are shown of murine tissues with increased macrophage (MΦ) infiltration upon CST knockout [CST-KO; C57BL/6 *ChgA*$^{del(1284-1346)/del(1284-1346)}$; 6000× magnification] [26]. Liver: hepatocytes (HC) and Kupfer cells (KC) with high MΦ infiltration are shown in the absence of CST (red). The increased infiltration was reversible upon intraperitoneal CST injection (green). Scale bar: 5 μm. Adrenal medulla: chromaffin cells (CC) with increased MΦ infiltration in CST-KO mice. Scale bar: 2 μm. Colon: increased bacterial infiltration, less diverse bacterial composition, and increased MΦ infiltration in CST-KO mice relative to wild-type mice. Scale bars: 2 μm. If translatable to humans, evidence from mice on the effects and localization of CST in inflammation might inform candidate therapeutic approaches relevant to humans. Abbreviations: MoC, monocyte; MV: microvilli. Figure created with BioRender (BioRender.com).

the sympathetic nervous system by binding to α- and β-adrenergic receptors. Its release increases blood pressure and heart rate, and reduces blood flow to the gastrointestinal system.

Paracrine signaling: cell produces a messenger molecule that triggers signaling in nearby cells.

heart, adrenal gland, and colon, compared with wild-type mice [26,30] (Figure 1). This increased macrophage infiltration has been associated with the hypertensive phenotype of these mutant mice; and macrophage depletion with clodronate liposomes in CST-KO mice normalized the blood pressure of these animals relative to controls [26]. However, this finding requires further validation with other approaches, because clodronate depletion does not fully remove other myeloid populations. Moreover, in mice with dextran sodium sulfate (DSS)-induced colitis, intraperitoneal injection of CST led to reduced concentrations of interleukin 1β (IL-1β), IL-6, and TNF-α in the colon, and peritoneal macrophages isolated from these mice produced smaller amounts of these cytokines [31]. Reduced macrophage numbers were reported in the liver of mice receiving a high-fat diet (DIO model) upon intraperitoneal CST injections [14], as well as in the heart of mice in the monogenic model of hypertension [26], the gut of mice with DSS-induced colitis [32], and atheromatous plaques of apolipoprotein E-deficient (*ApoE*$^{-/-}$) mice, compared with controls [33] (Figure 1). In addition, murine bone marrow-derived macrophages (BMDMs) and intraperitoneal macrophages cultured *in vitro* in the presence of CST exhibited skewed polarization toward an anti-inflammatory phenotype by expressing higher amounts of the anti-inflammatory cytokine IL-10 and lower amounts of IL-1β and TNF-α, CCL2, CCL3, and CXCL1 relative to untreated macrophages [26,31]. *In vivo* studies strengthen the presumed anti-inflammatory role of CST; for instance, relative to controls, bone marrow (BM) transfer from CST-KO mice to irradiated wild-type mice resulted in elevated amounts of IL-1β, TNF-α, CCL2, CCL3, and CXCL1, and lower amounts of IL-10 in the heart (the only organ assessed), and induced a hypertensive phenotype [26]. By contrast, BM transfer in the reverse direction (i.e., from wild-type to CST-KO mice) resulted in the opposite phenotypes, with low concentrations of IL-1β, TNF-α, CCL2, CCL3, and CXCL1, and elevated concentrations of IL-10 [26]. These experiments suggest that CST harbors immunomodulatory effects that can contribute to suppressing macrophage-driven inflammation, at least in these mouse models. Based on these findings, it is hypothesized that the co-release of CST together with catecholamines acts to dampen inflammation upon elevated neurohumoral activity [7].

A question is how CST signals in macrophages. One possibility is that this occurs via nicotinic acetylcholine receptors (nAChRs). Based on observations that CST inhibits norepinephrine release from PC12 cells only when triggered by nicotine (and not when secretion is triggered by ATP or other agents downstream of the nAChR pathway), it has been suggested that CST binds to nAChRs [16]. This has been confirmed using binding studies of CST to PC12 and cultured chromaffin cells, the assessment of covalent CST cross-linking to nAChR subunits [34], the electrophysiology of oocytes expressing different combinations of nAChR subunits, and by single-cell studies of acetylcholine-stimulated adrenal chromaffin cells [35]. Macrophages express nAChRs [36,37], and engagement of these receptors can result in anti-inflammatory effects. For example, nicotine and acetylcholine inhibit lipopolysaccharide (LPS)-induced production of TNF-α, IL-1β, IL-6, and IL-18 in human monocyte-derived macrophages [37]. Moreover, in isolated murine peritoneal macrophages, nicotine leads to the phosphorylation of signal transducer and activator of transcription 3 (STAT3), which inhibits LPS-induced production of TNF-α, CXCL2, and IL-6 [36]. Another possibility is that CST directly enters macrophages. Microscopy with fluorescently labeled CST showed that CST enters the cytosol of cultured human blood monocytes, polymorphonuclear leukocytes, and monocyte-derived dendritic cells and macrophages [38,39]. In fact, since cell entry was also observed at 4°C, where no endocytosis takes place, CST has been appreciated as a cell-penetrating peptide capable of passively entering cells [39]. Furthermore, CST surface plasmon resonance and affinity chromatography with purified calmodulin showed that CST in the cytosol directly binds to calmodulin and can trigger calcium signaling and calcium-independent phospholipase A2 (iPLA2) signaling via calmodulin [38]. Finally, since CST can exert certain metabolic effects (some described above), CST might also affect the immunometabolism of macrophages [40].

Macrophages might be an important source of CST

The BM transfer experiments discussed above [26] are relevant because wild-type mice receiving CST-KO BM transplants harbor completely autologous CST production from their neuroendocrine cells, while BM-derived cells from CST-KO mice receiving wild-type transplanted BM are affected and are the only source contributing to CST production (including macrophages). Indeed, the experiments suggested that macrophages (and/or other BM-derived cells) are major producers of CST themselves, although this remains conjectural. Nevertheless, the potential involvement of macrophages has been suggested by the following two observations: (i) the inflammatory and hypertensive phenotypes of CST-KO mice were fully reversible upon BM transfer from wild-type mice, showing that CST produced by BM-derived cells was sufficient to result in these phenotypes; and (ii) the concentration of CST in circulation in CST-KO mice was almost normalized following wild-type BM transfer [26]. Moreover, since BM transfer from CST-KO mice to wild-type mice induced inflammatory and hypertensive phenotypes, this suggested that CST was required to induce the observed physiological outcomes in this model, and that macrophages might be a relevant source of CST [26]. Macrophage CST production was confirmed by western blotting of lysates from isolated murine peritoneal macrophages [26], although CST production by contaminating cell types in this cell population cannot be ruled out and there is no evidence of macrophages directly producing CST at inflammation sites. Other open questions include assessing which subsets of monocytes and macrophages produce CST and which signals induce its production. Thus, although warranting further and robust investigation, we are intrigued by the conjecture that macrophages might be cells that are not only affected by CST, but also producers of it.

CST: a putative factor for macrophage self-suppression?

Thus, a key open question is investigating under which conditions macrophages produce CST. This is challenging to address, since the regulation of proteolytic processing of CgA to produce CST is not clear. There are multiple proteases that can lead to the production of CST: cathepsin-L [20], thrombin [41], furin [42], prohormone convertases 1 and 2 [42], kallikrein [43], and plasmin [44,45]. Several of these proteases have known roles in macrophages. For example, the lysosomal endopeptidase cathepsin L is highly expressed by macrophages, and culturing experiments with murine BMDMs suggested that this enzyme was implicated in the killing of pathogenic *Staphylococcus aureus* [46]. Moreover, upregulated expression of the proprotein convertase furin has been reported in activated macrophages compared with wild-type mice; LPS injection in mice harboring a conditional deletion of *Furin* in their myeloid cells ($LysM^{Cre\text{-}fur(fl/fl)}$) resulted in accelerated mortality, elevated serum concentrations of TNF-α and IL-6, and upregulated numbers of proinflammatory macrophages relative to wild-type mice [47].

Since several of the proteases that convert CgA into CST are involved in inflammatory processes, one possibility is that macrophages produce CST in an inflammation-dependent manner. In line with this, plasma CST concentrations have been reported to be elevated relative to healthy controls in patients with heart failure [48,49], end-stage renal disease [50], women with preeclampsia [51], sleep apnea [52], and acute pulmonary embolism [53]. Elevated concentrations of CST have also been measured in the blood of patients with inflammatory bowel disease [30,54], and these concentrations appear to correlate with disease severity [55]. However, plasma CST concentrations have been reported as decreased in patients with hypertension [56,57] and obesity [58] relative to healthy individuals and, therefore, such apparently contradictory findings need to be sorted out. In dogs, CST concentrations in the saliva have been correlated with cortisol concentrations in serum and stress behavior [59].

Clinician's corner

The prohormone CgA is proteolytically cleaved to produce the 21-amino acid peptide CST [7,8]. CST can suppress catecholamine release by neuroendocrine cells [16,19,26] and the inflammatory activation of macrophages [26,31].

Elevated CST concentrations in plasma have been associated with human diseases and disorders hallmarked by chronic inflammation, including type 2 diabetes mellitus [58], hypertension [56,57], heart failure [48,49], end-stage renal disease [50], preeclampsia [51], sleep apnea [52], acute pulmonary embolism [53], and inflammatory bowel disease [30,54,55].

In addition to neurons and neuroendocrine cells, macrophages can be important producers of CST [26]. CST produced by macrophages (or other bone marrow-derived cells) might contribute to its antiadrenergic effects [26].

Intraperitoneal injection of CST in mouse models of chronic inflammatory diseases can result in reduced macrophage infiltration and reduced amounts of circulating inflammatory cytokines, as observed in the liver of DOI mice [14], the heart of mice with ischemia-reperfusion injury [26], the colon of mice with DSS-induced colitis [31], the gut of mice with DSS-induced colitis [32], and the atheromatous plaques of $ApoE^{-/-}$ mice [33].

Following the hypothesis that macrophages might produce CST in an inflammation-dependent manner, we propose CST as a newly designated putative autocrine signaling factor for self-suppression of macrophage activation, although this warrants robust follow-up investigation.

Macrophage-produced CST might suppress neurotransmitter release from neuroendocrine cells

If CST produced by macrophages contributes to dampening inflammation, this also raises the question of what the contribution of CST produced by neuroendocrine cells might be on the neuroendocrine system. Might CST produced by neuroendocrine cells result in self-suppression of catecholamine release, in a similar fashion as previously envisioned for nicotine or pituitary adenylate-cyclase-activating polypeptide (PACAP)-induced catecholamine secretion [7,16,19,28]? Alternatively, might CST produced by macrophages contribute to regulating the activity of neurons and neuroendocrine cells? Recent evidence supports the latter, since BM transfer from wild-type mice into CST-KO mice resulted in normalization of the heightened norepinephrine and epinephrine concentrations in the plasma and adrenal gland, whereas BM transfer in the reverse direction resulted in elevated concentrations of these catecholamines [26].

Macrophage-produced CST might regulate both enteroendocrine cells and neurons in the intestine. As discussed above, CST has profound roles in the intestine, because CST-KO mice exhibit increased gut permeability and inflammation, both reversible by intraperitoneal CST injection [30]. Moreover, intrarectal CST administration can reduce inflammation in mice with DSS-induced colitis [31,32,55]. CST can also affect gut commensal bacterial populations, since intrarectal CST administration in wild-type mice reduced the *Firmicutes* to *Bacteroidetes* ratio [13], whereas this ratio was increased in CST-KO mice [30]. These processes are tightly regulated by neurotransmitters produced by enteroendocrine cells and enteric neurons, including catecholamine (reviewed in [60,61]). For example, exposure of isolated rat colon to catechol-*O*-methyltransferase inhibitors, impairing the degradation of catecholamines, suppressed longitudinal muscle contraction, decreasing colonic transit [62]. Additionally, vagotomy (scission of the vagus nerve connecting the brain to the intestine) worsened the severity of DSS-induced colitis in mice [63]. Given that the close contact between nerve fibers and macrophages in the colon can facilitate their reciprocal crosstalk mediated by neurotransmitters, cytokines, and hormones [36,64,65], it is reasonable to speculate that macrophage-derived CST signals to this system, thereby contributing to the regulation of gastrointestinal activity, which is an intriguing possibility (Figure 2, Key figure).

Concluding remarks

Based on the above discussion, we propose macrophages as a relevant source of CST that helps suppress macrophage-driven inflammation and catecholamine secretion in an inflammation-dependent fashion. However, many questions remain (see Outstanding questions) and supportive evidence is required to fully investigate these hypotheses. Indeed, a limitation of the current murine studies is the fact that global KO mice for CST were used. This model does not allow researchers to discern between CST produced by neurons, neuroendocrine cells, macrophages, or other myeloid populations. Indeed, cell-specific conditional and cell-specific CST-KO mice [e.g., using Cre recombinase/loxP models (Cre/loxP)] would be valuable tools to interrogate their contribution to CST production.

Moreover, conditional CST-KO mice might help to distinguish between locally versus systemically produced CST contributions and perhaps delineate the roles of CST in different organs. If suggesting plasma CST as a putative biomarker for inflammatory diseases, such as type 2 diabetes mellitus [58], hypertension [56,57], heart failure [48,49,66,67], sleep apnea [52] or acute pulmonary

Outstanding questions

Which proteases are responsible for the proteolytic production of CST? Their identification can inform on how CST production is regulated, which might help the assessment and design of putative treatments to restore/regulate its production.

How is the transcription/translation of CgA regulated? Can we identify the activity of the proteases that process CgA in macrophage-specific CST? Which subsets of monocytes and macrophages produce CST and which signals induce its production? Knowing the regulation of the production of CST might help increase our understanding of when and how CST contributes to suppressing inflammation. Perhaps such identified proteases might be considered as putative therapeutic targets in inflammatory scenarios.

How does CST exert its effects? What are the receptors for CST and how does it signal intracellularly? Information about the receptors and signaling pathways can help provide mechanistic insight into its function during homeostasis and inflammation.

What are the contributions of CST produced by neurons and neuroendocrine cells on catecholamine production and the immune system? Understanding the contribution of neuroendocrine CST might help us to understand the context in which it acts and its potential role affecting the interplay between neurons and the immune system in health and disease.

Does CST also exert anti-inflammatory effects on human macrophages? Do human macrophages also produce CST? CST has been mainly studied in mice. Thus, it needs to be determined how translatable the findings from the studies discussed here are to humans. Can CST-related pathways be realistically considered as potential therapeutic targets, and for which pathologies?

Key figure

Proposed model of catestatin (CST)-mediated communication between macrophages and the enteric neuroendocrine system

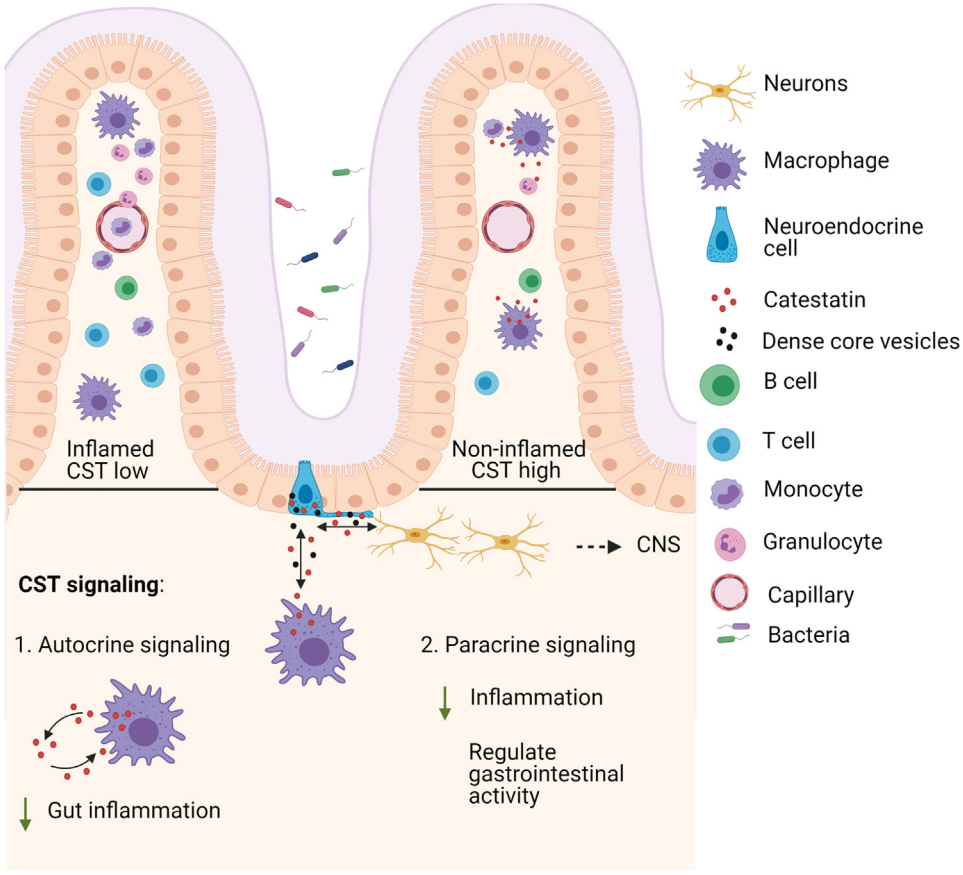

Figure 2. We propose that CST mediates the communication between macrophages, neuroendocrine cells, and enteric neurons, thereby contributing to the regulation of gastrointestinal activity and reducing inflammation. These effects might occur via: (1) autocrine signaling, in which CST is produced by macrophages, but also affects macrophages directly; and (2) paracrine signaling from macrophages to neuroendocrine cells. This proposed model remains to be tested and validated. Abbreviation: CNS, central nervous system. Figure created with BioRender (BioRender.com).

embolism [53], it will be important to fully understand these possible local CST functional roles, assuming they might affect the neuro–endocrine–immunological axis. As a complementary approach, the specific roles of CST in different organs might also be assessed by reducing the local amounts of CST in wild-type mice with injections of neutralizing antibodies. Alternatively, local CST concentrations might be increased by injecting (labeled or unlabeled) CST in distinct organs and distinct animal models and carefully evaluating functional and phenotypic outcomes.

Hypothetically, CST might be considered a candidate therapeutic target for treating patients with overactivated macrophages in specific diseases associated with inflammation. However, peptides are rapidly degraded in circulation and, in general, have a short half-life, not favoring

the direct administration of CST. The use of so-called retro-inverso-CST (RI-CST) might overcome this limitation [68]. This peptide contains D-isomer amino acids instead of L-isomers and has a reversed sequence; although, it is unclear whether receptor binding is identical to normal CST, RI-CST has been reported to rescue hypertension in CgA-KO mice for >8 h after intraperitoneal administration, whereas this rescue was only achieved for ~3 h using wild-type CST [68]. Thus, in contrast to normal CST, RI-CST is likely not efficiently broken down by proteases and, thus, might exhibit an extended half-life, even when administered orally, although this remains to be tested. From another angle, a pharmacophore-based screen identified the small organic compound TKO-10-18 as a potential drug that can mimic CST actions [69]. Thus, the administration of RI-CST or TKO-10-18 might be considered as putative therapies to be investigated preclinically for certain diseases associated with inflammatory and neuroendocrine dysfunctions, perhaps even metabolic disorders, or potentially autoimmune diseases, such as diabetes and inflammatory bowel disease, a possibility that certainly merits further attention.

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Declaration of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors contributions

E.M.M. and G.v.d.B. wrote the manuscript. S.K.M. provided the electron microscopy photographs used in Figure 1. G.C. and S.K.M. participated in discussing and editing of the manuscript.

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