Int J Clin Exp Med (2008) 1, 203-212
www.ijcem.com/IJCEM805003

Review Article

Cholinergic Modulation of Inflammation

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Received May 7, 2008; accepted May 30, 2008; available online June 5, 2008

Abstract: Recent studies have demonstrated that cytokine levels and inflammation can be regulated by specifically augmenting cholinergic signaling via the efferent vagus nerve and the α7 subunit-containing nicotinic acetylcholine receptor (α7nAChR). Cholinergic modalities, acting through vagus nerve- and/or α7nAChR-mediated mechanisms have been shown to suppress excessive inflammation in several experimental models of disease, including endotoxemic shock, sepsis, ischemia-reperfusion injury, hemorrhagic shock, colitis, postoperative ileus and pancreatitis. These studies have advanced the current understanding of the mechanisms regulating inflammation. They have also provided a rationale for exploring new possibilities to treat excessive, disease-underlying inflammation by applying selective cholinergic modalities in preclinical and clinical settings. An overview of this research is presented here.

Key Words: Inflammation, inflammatory diseases, cytokines, vagus nerve, α7nAChR, cholinergic anti-inflammatory pathway

Introduction

An imbalance in the highly regulated local inflammatory response to infection and injury can result in the excessive production of TNF, IL-1β, high mobility group box1 (HMGB1) and other inflammatory molecules by immune cells and their subsequent release into the circulation [1]. These systemic cytokine responses are associated with unrestrained inflammation, secondary tissue injury and diffuse coagulation underlying the pathology of septic shock, sepsis and other disorders [1,2]. Suppressing excessive pro-inflammatory cytokine production and function counteracts exacerbated inflammation and represents an important aspect of therapeutic strategies in the treatment of inflammatory diseases. Exploring TNF and other pro-inflammatory cytokines as therapeutic targets in preclinical and clinical settings is highly validated by the success of the anti-TNF approaches in the treatment of rheumatoid arthritis and Crohn's disease [3-5].

A physiological, neural mechanism that controls inflammation by suppressing the overproduction and systemic release of TNF and other pro-inflammatory cytokines has been identified by Kevin Tracey and colleagues [1,6-8]. The anti-inflammatory activity of this "cholinergic anti-inflammatory pathway" pathway is based on cholinergic signals through the efferent vagus nerve that are linked to macrophages and other innate immune cells through the α7 subunit-containing nicotinic acetylcholine receptor (α7nAChR) [6,7,9]. The cholinergic anti-inflammatory pathway has been a subject of extensive research for the last 10 years and vagus nerve stimulation and/or pharmacological activation of the α7nAChR have been utilized in several experimental models of disease. In addition, a role for brain cholinergic mechanisms in regulating systemic inflammation and their association with the vagaly-mediated cholinergic anti-inflammatory pathway have been indicated [10]. These studies have advanced our understanding of the cholinergic modulation of inflammation and present novel therapeutic modalities for controlling excessive inflammation that contributes to the pathogenesis of inflammatory diseases. An overview of

research related to cholinergic suppression of this disease-underlying inflammation is presented here.

Endotoxemia

A great deal of insight into the cholinergic mechanisms regulating inflammatory responses has been provided by utilizing the simple and reliable models of rat and murine endotoxemia. Acetylcholine suppresses endotoxin-stimulated macrophage release of TNF, IL-1β, IL-6 and IL-18 [6] and these observations have provided a rationale for in vivo studies exploring cholinergic regulation of endotoxin-generated systemic pro-inflammatory cytokine release. Administration of exogenous acetylcholine to endotoxemic animals is an approach that is limited by the rapid degradation of acetylcholine by acetylcholinesterases *in vivo*. Instead, the release of endogenous acetylcholine can be regulated by modulating the activity of the efferent vagus nerve, as acetylcholine is the principle neurotransmitter released by this nerve. Administration of endotoxin to rats results in elevated serum TNF levels. Surgical transection of the vagus nerve (vagotomy) exacerbates this systemic cytokine response, thus indicating that the vagus nerve exercises a tonic control on cytokine production and/or release [6]. Electrical stimulation of the distal end of the vagus nerve, after vagotomy, lowers serum and organ TNF levels [6], revealing that activation of efferent vagus nerve activity results in an anti-inflammatory effect. By contrast, circulating levels of IL-10, an anti-inflammatory cytokine are not changed after electrical stimulation of the vagus nerve. This observation is consistent with the lack of effect of acetylcholine on IL-10 release from endotoxin-stimulated macrophages [6]. These findings have provided insight into the role of the vagus nerve in controlling excessive inflammation: while endogenous vagal activity exerts tonic anti-inflammatory effects, its augmentation (by electrical stimulation) is necessary to counteract exacerbated pro-inflammatory cytokine release. In addition to aberrant cytokine responses, rats subjected to lethal endotoxemia also develop severe hypotension and shock. Vagus nerve stimulation rescues animals from the development of these complications [6]. Recently, the suppressive effect of electrical vagus nerve stimulation on serum TNF levels was recapitulated by transcutaneous vagus nerve stimulation [11]. This non-invasive approach of vagus nerve stimulation potentially could be developed for the treatment of pathological conditions characterized by acute or chronic inflammation.

These findings demonstrate the anti-inflammatory function of the efferent vagus nerve, which is the major parasympathetic nerve, originating from the brainstem medulla oblongata. The brain origin of the efferent vagus nerve and previous knowledge that vagus nerve functions are modulated by brain regions located above the brainstem indicated the possibility that the anti-inflammatory function of the vagus nerve can be centrally regulated. A role for afferent vagal neurons in modulating inflammation has also been demonstrated; cytokines and other inflammatory products found in the periphery can activate afferent vagus nerve signaling that reaches the nucleus tractus solitarius and other "higher" brain structures [12]. Thus, vagal afferents can serve as sensors of peripheral inflammation that signal the brain to respond with sickness behavior and immunomodulatory output [12]. Vagal afferent and efferent neurons form a reflex mechanism that modulates inflammation and counteracts the adverse effects of cytokine overproduction [13]. The efferent arm of this inflammatory reflex, the cholinergic anti-inflammatory pathway, can be centrally activated as initially shown using the tetravalent guanylhydrazone CNI-1493 [14]. This compound, which crosses the blood brain barrier, inhibits serum and organ TNF levels following central (i.c.v.) or peripheral (i.p.) administration and protects animals against the development of endotoxin-induced shock [14]. The anti-inflammatory activity of CNI-1493 requires efferent vagus nerve signaling because its effect is abolished in vagotomized rats [14]. Brain mechanisms that link the effect of CNI-1493 to suppression of TNF levels through vagus nerve signaling are currently unknown. Interestingly, this compound also has been shown to interact with muscarinic acetylcholine receptors [10], and we have shown that muscarinic receptor ligands, administered centrally, significantly suppress serum TNF levels during endotoxemia [10]. Central (i.c.v.) administration of the M1 receptor agonist McN-A-343 dose-dependently reduces serum TNF levels, thus indicating a role for the CNS M1 subtype in the modulation of endotoxin-

induced pro-inflammatory cytokine release [10]. Moreover, i.c.v. administration of the M2 antagonist, methoctramine, dose-dependently decreases serum TNF levels [10]. The brain M2 receptor functions in the cholinergic synapse as a pre-synaptic acetylcholine autoreceptor, mediating the inhibition of acetylcholine release [15]. In view of this knowledge, our finding indicates that unblocking this inhibition by methoctramine [16] results in an anti-inflammatory effect in the periphery [10]. The i.c.v. administration of methoctramine to rats also stimulates efferent vagus nerve activity, as demonstrated by increased high frequency power component of heart rate variability in rats, an indicator of vagal regulation of the heart [10]. In contrast to brain muscarinic receptors, it appears that peripheral muscarinic receptors do not play a major role in transmitting anti-inflammatory cholinergic output, because blocking these receptors by administration of atropine methyl nitrate does not reverse the suppression of serum TNF levels by vagus nerve stimulation in endotoxemic rats [10]. Revealing the brain mechanisms involved in the regulation of inflammation and their relationship with the vagus nerve-dependent cholinergic anti-inflammatory pathway will facilitate the development of new centrally-acting anti-inflammatory agents.

The initial observation that acetylcholine suppresses the release of pro-inflammatory cytokines has facilitated research aimed at identifying mediating receptor mechanisms. Muscarinic and nicotinic acetylcholine receptors are distributed in the CNS, peripheral nervous system and neuroeffector cells, including muscle cells, glandular cells and cardiac miocytes. Several muscarinic receptor subtypes and nicotinic receptor subunits also have been identified on cytokine-producing cells, including macrophages, dendritic cells, mast cells and endothelial cells [7,17,18]. Nicotine, a prototype nicotinic acetylcholine receptor agonist, causes a dose-dependent reduction in TNF release from endotoxin-stimulated human macrophages, which is more-efficient than acetylcholine [6]. Muscarine, a prototype agonist of muscarinic receptors is much less efficient than acetylcholine [6]. These observations, together with the fact that macrophages are major producers of TNF during endotoxemia, suggest that nicotinic receptors on macrophages and other immune cells are involved in the cholinergic regulation of inflammation *in vivo*. Macrophages have been shown to express functional α1, α7, and α10 subunits of nicotinic receptors [7]. Importantly, an antisense oligonucleotides to the α7 subunit abolishes nicotine suppression of endotoxin-stimulated TNF release from human macrophages [7]. In contrast, antisense oligonucleotides to the α1 and α10 subunits, under similar conditions do not significantly alter TNF release in the presence of nicotine [19]. These data demonstrate the critical role of the α7nAChR in mediating the suppressive effect of nicotine on TNF release *in vitro*. Experiments with α7nAChR KO mice reveal the importance of the α7nAChR in mediating the cholinergic anti-inflammatory efficacy of vagus nerve stimulation [7]. Serum levels of TNF, IL-1, and IL-6 in α7nAChR KO mice are significantly higher during endotoxemia than the cytokine levels in the wild type animals [7]. Vagus nerve stimulation reduces serum TNF levels in wild type animals subjected to endotoxemia, but does not significantly alter cytokine levels in α7nAChR KO mice [7]. In addition, nicotine is ineffective in suppressing TNF release from endotoxin-activated peritoneal macrophages isolated from α7nAChR KO mice [7]. Taken together these findings demonstrate the importance of the α7nAChR as a component of the cholinergic regulation of inflammation. In addition these findings indicate that this receptor can be targeted by specific agonists to control excessive inflammatory responses. An example of an α7nAChR agonist is GTS-21, a compound previously used to counteract cognitive deterioration in preclinical studies of Alzheimer's disease. GTS-21 is considerably less toxic than nicotine and is well tolerated by human volunteers. Therefore, GTS- 21 is a good candidate for mechanistic studies and further therapeutic development. GTS-21 inhibits TNF release from endotoxin-stimulated RAW 264.7 macrophages and suppresses the activation of the NF-κB, a transcription factor with a major role in TNF synthesis [20]. Administration of GTS-21 also dose-dependently suppresses serum TNF and improves survival during lethal endotoxemia [20].

The organ specificity of the cholinergic regulation of cytokine responses during endotoxemia has been recently highlighted by studies showing that the spleen is a major source of TNF and critically important for the

anti-inflammatory functioning of the cholinergic pathway [21]. Vagus nerve stimulation suppresses splenic TNF levels in wild type mice, but failed to significantly alter splenic TNF levels in α7nAChR KO mice, thus indicating that an α7nAChR-mediated mechanism is involved in this anti-inflammatory effect. Vagus nerve stimulation also suppresses hepatic and heart TNF levels, but has no effect on lung TNF levels [14].

Sepsis

Recent studies indicate the role of the α7nAChR and the vagus nerve in regulating inflammation during experimental sepsis and the beneficial therapeutic effect of α7nAChR agonists or vagus nerve stimulation. Suppressing excessive inflammation associated with polymicrobial sepsis at relatively late stages of this disorder is an important therapeutic approach. HMGB1 is a late mediator of inflammation following the earlier "cytokine storm" caused by TNF, IL-1β and other early inflammatory mediators of sepsis [22]. Neutralizing the deleterious activity or suppressing the production of HMGB1 improves survival in preclinical models of sepsis even when the first treatment is initiated 24h after the onset of the disease thus validating the critical role of HMGB1 in mediating sepsis lethality [19, 23].

The release of HMGB1 from endotoxin-stimulated macrophages can be suppressed by acetylcholine and nicotine, acting through an α7nAChR-mediated mechanism [19]. The α7nAChR selective agonist GTS-21 also inhibits HMGB1 release from RAW macrophages [20]. These effects of nicotine and GTS-21 occur in parallel with significant suppression of NF-κB activation [19, 20]. Treatment with nicotine or GTS-21 initiated 24h after polymicrobial sepsis induced by cecal ligation and puncture (CLP) surgery and continued for 3 days results in significant survival improvement, indicating the possibility of treating sepsis within a clinically-relevant time frame [19,20]. Interestingly, nicotine administration fails to suppress HMGB1 levels and improve survival in splenectomized mice; serum HMGB1 levels and mortality are even higher in splenectomized animals treated with nicotine [21]. These findings indicate that the spleen is required for the anti-inflammatory activity of cholinergic stimulation.

A role for the vagus nerve in regulating inflammation during sepsis has also been demonstrated. Vagus nerve stimulation applied as a directed transcutaneous manipulation, starting 24h after surgery suppresses serum HMGB1 levels and improves survival in mice following CLP-induced sepsis [11]. This study demonstrates the beneficial effect of a non-invasive form of vagus nerve stimulation for the treatment of preclinical sepsis that also is associated with HMGB1 suppression.

Recently Wu et al [24] demonstrate that peripheral administration of ghrelin to rats subjected to CLP-induced sepsis, significantly inhibits serum TNF and IL-6 levels. Ghrelin is a gastric hormone with multiple autocrine, paracrine and endocrine effects. Ghrelin crosses the blood brain barrier and some of its physiological functions are CNS mediated [25]. Ghrelin has also been shown to increase efferent vagus nerve activity when administered i.c.v. [26]. The anti- inflammatory efficacy of ghrelin in sepsis requires an intact vagus nerve because bilateral subdiaphragmatic vagotomy abolishes the suppression. The primary role of the vagus nerve in mediating the anti-inflammatory efficacy of ghrelin is further supported by the lack of a direct suppressive effect of ghrelin on TNF and IL-6 release from endotoxin-stimulated Kupffer cells or peritoneal macrophages.

Ischemia-reperfusion injury

Reperfusion injury after ischemia is a severe complication that is associated with the systemic release of toxic compounds when the blood flow is restored. The release of TNF and other pro-inflammatory cytokines plays an important role in ischemia-reperfusion injury by mediating myocardial depression, severe hypotension and multiple organ failure. Bernik et al [27] have shown that electrical vagus nerve stimulation suppresses serum and organ TNF levels in rats with ischemia-reperfusion caused by aortic occlusion. Vagus nerve stimulation also significantly protects animals against the development of hypotension and shock [27].

Recently, Yeboah et al [28] have reported the protective effects of cholinergic modalities against renal ischemia-reperfusion injury. The authors show that kidney cells express

nicotinic receptors, including a functional α7nAChR and the α7nAChR agonist nicotine and GTS-21 reduce ischemia-induced kidney TNF levels and leukocyte trafficking to the kidney. Nicotine and GTS-21 also suppress kidney damage as determined by serum creatinine levels and tubular epithelial cell necrosis. Bilateral subdiaphragmatic vagotomy does not alter the effects of nicotine in this model of renal ischemia-reperfusion injury in rats, suggesting a local effect of cholinergic agonists. This finding is related to the intriguing possibility that cholinergic modalities can be utilized in the treatment of complications caused by ischemia in transplanted kidney, lacking vagal innervations.

In a recent study Wu et al [29] have described a critical role of vagus nerve signaling in mediating the protective effects of ghrelin in a rat model of intestinal ischemia-reperfusion injury caused by superior mesenteric artery occlusion. This study demonstrates that gut ischemia-reperfusion is associated with markedly lower serum levels of ghrelin and elevated pro-inflammatory cytokine levels. Accordingly, administration of ghrelin lowers serum TNF and IL-6 levels and suppresses neutrophil infiltration. Moreover, exogenous ghrelin has protective effects against organ injury and mortality. Vagotomy abrogates beneficial effects of ghrelin, indicating an important mediating role for vagus nerve signals. Ghrelin also causes beneficial effects when administered i.c.v. This finding together with the reported distribution of brain ghrelin receptors indicates that ghrelin may centrally activate the cholinergic anti-inflammatory pathway to suppress excessive inflammation and protect against organ damage and mortality during gut ischemia-reperfusion in rats.

Hemorrhagic shock

The severe complications of hemorrhagic shock resulting from trauma are characterized by cell and organ injury and abnormal cardiovascular and systemic inflammatory responses [30]. Guarini et al. [31,32] demonstrated that electrical vagus nerve stimulation reverses hypotension and increases the survival time of rats subjected to acute hypovolemic hemorrhagic shock [31]. The vagus nerve also plays a role in regulating the inflammatory response during acute hypovolemic hemorrhagic shock. Vagus nerve stimulation inhibits the hepatic NF-κB activation by blocking the degradation of I-κB, and decreases both hepatic TNF mRNA expression and serum TNF levels [31]. Treatment with the nicotinic acetylcholine receptor antagonist chlorisondamine abrogates these effects of vagus nerve stimulation [31]. In another study Guarini et al. [32] reported that melanocortin ACTH (1-24) restores cardiovascular and respiratory function and prevents mortality and these effects are associated with increased efferent vagus nerve activity, inhibition of the hepatic NF-κB activation and decreased hepatic TNF mRNA expression and serum TNF levels. The effects of ACTH (1-24) are abolished by bilateral cervical vagotomy, inhibition of central MC4 or central muscarinic receptors, and peripheral nicotinic receptors, thus demonstrating a mediating role for cholinergic mechanisms [32].

Luyer et al. [33] showed that the inflammatory reflex can be regulated through ingestion of a high fat diet. This study demonstrates that high fat enteral nutrition causes suppression of systemic TNF and IL-6 levels and prevents the loss of intestinal barrier integrity in rats subjected to non-lethal hemorrhagic shock [33]. The authors also show that these effects are attributed to the release of cholecystokinin that activates both central and peripheral cholecystokinin receptors [33]. Subdiaphragmatic vagotomy or a pretreatment with the nicotinic receptor antagonist chlorisondamine prior to hemorrhagic shock abolishes the anti-inflammatory efficacy of the high fat nutrition, thus suggesting the mediating role of afferent and efferent vagus nerve signaling and peripheral nicotinic receptors that form the inflammatory reflex [33]. The authors also propose that a tonic anti-inflammatory function of the efferent vagus nerve may significantly contribute to maintain the intestinal hyporesponsiveness to dietary antigens [33].

Pancreatitis

Van Westerloo at al [34] have demonstrated the cholinergic suppression of cerulean-induced pancreatitis severity and inflammation in mice. Treatment with the selective α7nAChR agonist GTS-21 significantly reduces pacreatitis severity as indicated by lower plasma amylase and lipase levels and protects

against pancreatic tissue damage, determined by improved pancreatic histology scores [34]. GTS-21 treatment also suppresses neutrophil influx into the pancreas, and reduces plasma IL-6 levels, as compared with saline- treated pancreatic animals [34]. In addition, unilateral cervical vagotomy or pretreatment with the nicotinic receptor antagonist mecamylamine exacerbates pancreatic damage and augmentes systemic IL-6 levels, thus indicating a role for the vagus nerve and nicotinic receptors in the regulation of pancreatitis [34]. Interestingly, this study shows that lung inflammation associated with pancreatitis is not affected by nicotinic agonist/antagonist treatments or vagotomy [34], which is consistent with the previous observation that vagus nerve stimulation does not suppress pro-inflammatory cytokine levels in the lungs [14].

Post-operative ileus

Manipulation-induced delay in gastric empting and intestinal inflammation, mediated by intestinal muscle layer resident macrophage activation are important characteristics of post-operative ileus [35, 36]. de Jonge et al. [37] showed that electrical vagus nerve stimulation suppresses intestinal inflammation and prevents the development of post-operative illeus in mice. The authors provide evidence that the underlying mechanisms involve activation of the signal transducer and activator of transcription (STAT) in intestinal macrophages [37]. In addition, nicotine suppresses TNF, macrophage inflammatory protein 2 (MIP-2), and IL-6 release from peritoneal macrophages via α7nAChR - mediated activation of the janus kinase (JAK)/STAT pathway [37]. In another recent study The et al have shown that treatment of mice with the α7nAChR agonist AR-R17779 abolishes delayed gastric empting caused by surgery intestinal manipulation and prevents post-operative ileus [38]. This compound also reduces pro- inflammatory cytokine release from peritoneal macrophages and suppresses NF-κB activation [38].

Colitis

Inflammatory bowel diseases, including ulcerative colitis and Crohn's disease are characterized by chronic intestinal inflammation that is mediated by TNF and other pro-inflammatory factors released from activated macrophages and other immune cells [39]. Recently Ghia et al. [40] demonstrated the protective, anti-inflammatory function of the vagus nerve in murine models of acute colitis and a role for nicotinic receptors and macrophages in mediating this function. Subdiaphragmatic vagotomy of mice prior to dextran sodium sulfate (DSS) - or hapten-induced colitis results in higher disease activity indices, elevated macroscopic and histological scores and increased colonic levels of TNF and other pro-inflammatory cytokines as compared to sham-operated mice. In addition, nicotine treatment reduces these parameters in vagotomized mice with colitis and hexamethonium (a nicotinic receptor antagonist) treatment exacerbates them in sham-operated animals subjected to DSS-induced colitis [40]. Vagotomy does not affect disease severity in M-CSF-deficient mice, thus indicating that the effect of vagotomy is macrophage-dependent [40]. The protective effect of the vagus nerve appears to be more prominent against acute colitis, because it is diminished when colitis is induced at more than 20 days post vagotomy [41]. However, the vagus nerve exerts tonic anti-inflammatory effect against acute colitis relapses on the background of existing chronic inflammation [42]. These findings together with the indicated autonomic imbalance, associated with impaired parasympathetic activity in inflammatory bowel diseases provide a rationale for new treatments of acute relapses of colitis based on activating the cholinergic anti-inflammatory pathway [39].

Subcutaneous inflammation

Saeed et al. [18] showed the cholinergic regulation of endothelial cell activation and leukocyte trafficking to a subcutaneous site of inflammation. TNF-induced endothelial cell activation is significantly suppressed by acetylcholine and other nicotinic receptor agonists and the nicotinic receptor antagonist, mecamylamine, abolishes these suppressive effects. In addition, nicotine inhibits NF-κB activation in these cells [18]. These in vitro studies indicate that the endothelial function during inflammation can be altered by cholinergic, nicotinic receptor-mediated mechanisms, which is consistent with the expression of the α7nAChR by endothelial cells [18]. Accordingly, nicotine and the novel nicotinic receptor agonist, CAP-55, dose-

dependently inhibit leukocyte recruitment in vivo using the carrageenan air pouch model in mice. Mecamylamine pretreatment abrogates the effect of nicotine and CAP-55, implicating cholinergic, nicotinic receptor mechanisms [18]. Moreover, electrical vagus nerve stimulation significantly suppresses leukocyte trafficking in this model [18]. These findings reveal the role of the cholinergic anti-inflammatory pathway in regulating leukocyte migration across the endothelium during local subcutaneous inflammation [18].

Human studies

Studies with rodents have revealed the anti-inflammatory potential of selective cholinergic stimulation in the treatment of diseases and pathological conditions characterized by excessive inflammation. A correlation between higher vagal tone as determined by increased high frequency component of heart rate variability and lower pro-inflammatory cytokine levels has also been demonstrated [10]. Autonomic dysfunctions, characterized by lower vagal and higher sympathetic tone have been documented in chronic inflammatory diseases and aging, associated with certain level of systemic inflammation [1, 43, 44]. These findings recently have been highlighted by the intriguing correlation between heart rate variability and serum levels of counteract excessive inflammation in humans. In addition to its important involvement in mediating sepsis pathology, the pro-inflammatory cytokine HMGB1 has a pathological role in rheumatoid arthritis [45]. Goldstein et al. [46] report that elevated serum HMGB1 and C-reactive protein (CRP) levels in patients with the disease, as compared with controls, are negatively correlated with heart rate variability, indicating a lower vagal tone. The disease activity scores also correlate with serum HMGB1 levels in the patients studied [46]. Sloan et al. have shown an inverse relationship between cardiac measures of vagus nerve activity and serum CRP and IL-6 levels in humans [47]. In a recent study by Marsland et al. [48], higher vagal tone, as determined by increased high frequency power component of heart rate variability, is associated with lower pro-inflammatory cytokine responses. Lower endotoxin-stimulated TNF and IL-6 release by peripheral blood monocytes or leukocytes *ex vivo* has been found in humans with higher vagal tone. Interestingly, no significant change in IL-10 release with respect to vagal tone differences has been reported [48], which is consistent with the lack of effect of the efferent vagus nerve on the serum levels of this anti-inflammatory cytokine during endotoxemia [6]. Although it is unclear whether these elevated inflammatory responses are due to lower vagal activity, it is plausible that activation of vagus nerve cholinergic outflow stimulators may be beneficial for improving the autonomic dysfunction and may have clinical implications in treating rheumatoid arthritis and other inflammatory disorders. Vagus nerve stimulators are already in clinical use for the treatment of epilepsy and depression. These clinically approved devices chronically activate afferent vagal signaling that affects brain function. In contrast, vagus nerve stimulators for treating inflammation should be designed to augment efferent vagus nerve anti-inflammatory signaling. The anti-inflammatory effects of GTS-21 and other selective α7nAChR agonists in preclinical studies and the information available for the safety profile of some of these agents in humans [49] can facilitate testing their anti-inflammatory efficacy in clinical settings. Centrally-acting cholinergic agents may also be utilized in treating inflammatory conditions in humans. It is important to test these and other intriguing and even provocative possibilities such as the role of cholinergic modalities in preventing and treating cancer [50, 51], in future studies.

Conclusions

Ongoing research has revealed that selective cholinergic activation through efferent vagus nerve stimulation or cholinergic modalities targeting the α7nAChR has a critical role in regulating cytokine responses and inflammation. Vagus nerve stimulation or α7nAChR agonists have been utilized in the treatment of excessive inflammation in pre-clinical models of disease, providing a rationale for the clinical development of these approaches.

Acknowledgements

This work was supported by The Feinstein Institute for Medical Research Award Program (to V.A.P.), North Shore-Long Island Jewish General Clinical Research Center, MO1 RR018535, and the National Institute of General Medical Sciences (to Kevin J. Tracey). The author thanks Dr Kevin J. Tracey and Dr

Christine N. Metz for their comments and suggestions. The author apologizes to those authors whose work on the cholinergic modulation of inflammation could not be cited.

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References

[1] Tracey KJ. Physiology and immunology of the cholinergic antiinflammatory pathway. J Clin Invest 2007; 117(2):289-296.

[2] Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, Hariri RJ, Fahey TJ, III, Zentella A, Albert JD, . Shock and tissue injury induced by recombinant human cachectin. Science 1986; 234(4775):470-474.

[3] Ghezzi P, Cerami A. Tumor necrosis factor as a pharmacological target. Mol Biotechnol 2005; 31(3):239-244.

[4] Kievit W, Fransen J, Oerlemans AJ, Kuper HH, van der Laar MA, de Rooij DJ, De Gendt CM, Ronday KH, Jansen TL, van Oijen PC, Brus HL, Adang EM, van Riel PL. The efficacy of anti-TNF in rheumatoid arthritis, a comparison between randomised controlled trials and clinical practice. Ann Rheum Dis 2007; 66(11):1473-1478.

[5] Sandborn WJ. Strategies for targeting tumour necrosis factor in IBD. Gastroenterol 2003; 17(1):105-117.

[6] Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 2000; 405(6785):458-462.

[7] Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, Li JH, Wang H, Yang H, Ulloa L, Al Abed Y, Czura CJ, Tracey KJ. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 2003; 421(6921):384-388.

[8] Pavlov VA, Wang H, Czura CJ, Friedman SG, Tracey KJ. The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. Mol Med 2003; 9(5-8):125-134.

[9] Gallowitsch-Puerta M, Pavlov VA. Neuro-immune interactions via the cholinergic anti-inflammatory pathway. Life Sci 2007; 80(24-25):2325-2329.

[10] Pavlov VA, Ochani M, Gallowitsch-Puerta M, Ochani K, Huston JM, Czura CJ, Al Abed Y, Tracey KJ. Central muscarinic cholinergic regulation of the systemic inflammatory response during endotoxemia. Proc Natl Acad Sci USA 2006; 103(13):5219-5223.

[11] Huston JM, Gallowitsch-Puerta M, Ochani M, Ochani K, Yuan R, Rosas-Ballina M, Ashok M, Goldstein RS, Chavan S, Pavlov VA, Metz CN, Yang H, Czura CJ, Wang H, Tracey KJ. Transcutaneous vagus nerve stimulation reduces serum high mobility group box 1 levels and improves survival in murine sepsis. J Exp Med 2007; 35(12):2762-2768.

[12] Goehler LE, Gaykema RP, Hansen MK, Anderson K, Maier SF, Watkins LR. Vagal immune-to-brain communication: a visceral chemosensory pathway. Autonom Neurosc 2000; 85(1-3):49-59.

[13] Tracey KJ. The inflammatory reflex. Nature 2002; 420(6917):853-859.

[14] Bernik TR, Friedman SG, Ochani M, DiRaimo R, Ulloa L, Yang H, Sudan S, Czura CJ, Ivanova SM, Tracey KJ. Pharmacological stimulation of the cholinergic antiinflammatory pathway. J Exp Med 2002; 195(6):781-788.

[15] Zhang W, Basile AS, Gomeza J, Volpicelli LA, Levey AI, Wess J. Characterization of central inhibitory muscarinic autoreceptors by the use of muscarinic acetylcholine receptor knock-out mice. J Neurosc 2002; 22(5):1709-1717.

[16] Stillman MJ, Shukitt-Hale B, Kong RM, Levy A, Lieberman HR. Elevation of hippocampal extracellular acetylcholine levels by methoctramine. Brain Res Bull 1993; 32(4):385-389.

[17] Kawashima K, Yoshikawa K, Fujii YX, Moriwaki Y, Misawa H. Expression and function of genes encoding cholinergic components in murine immune cells. Life Sci 2007; 80(24-25):2314-2319.

[18] Saeed RW, Varma S, Peng-Nemeroff T, Sherry B, Balakhaneh D, Huston J, Tracey KJ, Al Abed Y, Metz CN. Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation. J Exp Med 2005; 201(7):1113-1123.

[19] Wang H, Liao H, Ochani M, Justiniani M, Lin X, Yang L, Al Abed Y, Wang H, Metz C, Miller EJ, Tracey KJ, Ulloa L. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. Nat Med 2004; 10(11):1216-1221.

[20] Pavlov VA, Ochani M, Yang LH, Gallowitsch-Puerta M, Ochani K, Lin X, Levi J, Parrish WR, Rosas-Ballina M, Czura CJ, Larosa GJ, Miller EJ, Tracey KJ, Al Abed Y. Selective alpha7-nicotinic acetylcholine receptor agonist GTS-21 improves survival in murine endotoxemia and severe sepsis. Crit Care Med 2007; 35(4):1139-1144.

[21] Huston JM, Ochani M, Rosas-Ballina M, Liao H, Ochani K, Pavlov VA, Gallowitsch-Puerta M, Ashok M, Czura CJ, Foxwell B, Tracey KJ, Ulloa L. Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. J Exp Med 2006; 203(7):1623-1628.

[22] Wang H, Bloom O, Zhang M, Vishnubhakat JM,

Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, Manogue KR, Faist E, Abraham E, Andersson J, Andersson U, Molina PE, Abumrad NN, Sama A, Tracey KJ. HMG-1 as a late mediator of endotoxin lethality in mice. Science 1999; 285(5425):248-251.

[23] Yang H, Ochani M, Li J, Qiang X, Tanovic M, Harris HE, Susarla SM, Ulloa L, Wang H, DiRaimo R, Czura CJ, Wang H, Roth J, Warren HS, Fink MP, Fenton MJ, Andersson U, Tracey KJ. Reversing established sepsis with antagonists of endogenous high-mobility group box 1. Proc Natl Acad Sci USA 2004; 101(1):296-301.

[24] Wu R, Dong W, Cui X, Zhou M, Simms HH, Ravikumar TS, Wang P. Ghrelin down-regulates proinflammatory cytokines in sepsis through activation of the vagus nerve. Ann Surg 2007; 245(3):480-486.

[25] Chen HY, Trumbauer ME, Chen AS, Weingarth DT, Adams JR, Frazier EG, Shen Z, Marsh DJ, Feighner SD, Guan XM, Ye Z, Nargund RP, Smith RG, Van der Ploeg LH, Howard AD, MacNeil DJ, Qian S. Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. Endocrinology 2004; 145(6):2607-2612.

[26] Sato N, Kanai S, Takano S, Kurosawa M, Funakoshi A, Miyasaka K. Central administration of ghrelin stimulates pancreatic exocrine secretion via the vagus in conscious rats. Jpn J Physiol 2003; 53(6):443-449.

[27] Bernik TR, Friedman SG, Ochani M, DiRaimo R, Susarla S, Czura CJ, Tracey KJ. Cholinergic antiinflammatory pathway inhibition of tumor necrosis factor during ischemia reperfusion. J Vasc Surg 2002; 36(6):1231-1236.

[28] Yeboah MM, Xue X, Duan B, Ochani M, Tracey KJ, Susin M, Metz CN. Cholinergic agonists attenuate renal ischemia-reperfusion injury in rats. Kidney Int 2008

[29] Wu R, Dong W, Ji Y, Zhou M, Marini CP, Ravikumar TS, Wang P. Orexigenic hormone ghrelin attenuates local and remote organ injury after intestinal ischemia-reperfusion. PLoS ONE 2008; 3(4):e2026-

[30] Wu R, Wang P. Preclinical studies with adrenomedullin and its binding protein as cardiovascular protective agents for hemorrhagic shock. Cardiovasc Drug Rev 2006; 24(3-4):204-213.

[31] Guarini S, Altavilla D, Cainazzo MM, Giuliani D, Bigiani A, Marini H, Squadrito G, Minutoli L, Bertolini A, Marini R, Adamo EB, Venuti FS, Squadrito F. Efferent vagal fibre stimulation blunts nuclear factor-kappaB activation and protects against hypovolemic hemorrhagic shock. Circulation 2003; 107(8):1189-1194.

[32] Guarini S, Cainazzo MM, Giuliani D, Mioni C, Altavilla D, Marini H, Bigiani A, Ghiaroni V, Passaniti M, Leone S, Bazzani C, Caputi AP, Squadrito F, Bertolini A. Adrenocorticotropin reverses hemorrhagic shock in anesthetized rats through the rapid activation of a vagal anti-inflammatory pathway. Cardiovasc Res 2004; 63(2):357-365.

[33] Luyer MD, Greve JW, Hadfoune M, Jacobs JA, Dejong CH, Buurman WA. Nutritional stimulation of cholecystokinin receptors inhibits inflammation via the vagus nerve. J Exp Med 2005; 202(8):1023-1029.

[34] van Westerloo DJ, Giebelen IA, Florquin S, Bruno MJ, Larosa GJ, Ulloa L, Tracey KJ, van der PT. The vagus nerve and nicotinic receptors modulate experimental pancreatitis severity in mice. Gastroenterology 2006; 130(6):1822-1830.

[35] Kalff JC, Schraut WH, Simmons RL, Bauer AJ. Surgical manipulation of the gut elicits an intestinal muscularis inflammatory response resulting in postsurgical ileus. Ann Surg 1998; 228(5):652-663.

[36] Livingston EH, Passaro EP, Jr. Postoperative ileus. Dig Dis Sci 1990; 35(1):121-132.

[37] de Jonge WJ, van der Zanden EP, The FO, Bijlsma MF, van Westerloo DJ, Bennink RJ, Berthoud HR, Uematsu S, Akira S, van den Wijngaard RM, Boeckxstaens GE. Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway. Nat Immunol 2005; 6(8):844-851.

[38] The FO, Boeckxstaens GE, Snoek SA, Cash JL, Bennink R, Larosa GJ, van den Wijngaard RM, Greaves DR, de Jonge WJ. Activation of the cholinergic anti-inflammatory pathway ameliorates postoperative ileus in mice. Gastroenterology 2007; 133(4):1219-1228.

[39] Bonaz B. The cholinergic anti-inflammatory pathway and the gastrointestinal tract. Gastroenterology 2007; 133(4):1370-1373.

[40] Ghia JE, Blennerhassett P, Kumar-Ondiveeran H, Verdu EF, Collins SM. The vagus nerve: a tonic inhibitory influence associated with inflammatory bowel disease in a murine model. Gastroenterology 2006; 131(4):1122-1130.

[41] Ghia JE, Blennerhassett P, Collins SM. Vagus nerve integrity and experimental colitis. Am J Physiol Gastrointest Liver Physiol 2007; 293(3):G560-G567.

[42] Ghia JE, Blennerhassett P, El Sharkawy RT, Collins SM. The protective effect of the vagus nerve in a murine model of chronic relapsing colitis. Am J Physiol Gastrointest Liver Physiol 2007; 293(4):G711-G718.

[43] Evrengul H, Dursunoglu D, Cobankara V, Polat B, Seleci D, Kabukcu S, Kaftan A, Semiz E, Kilic M. Heart rate variability in patients with rheumatoid arthritis. Rheumatol Int 2004; 24(4):198-202.

[44] Muller-Werdan U. Inflammation and ageing. Z Gerontol Geriatr 2007; 40(5):362-365.

[45] Wang H, Li W, Goldstein R, Tracey KJ, Sama AE. HMGB1 as a potential therapeutic target. Novartis Found Symp 2007; 280:73-85.

[46] Goldstein RS, Bruchfeld A, Yang L, Qureshi AR,

Gallowitsch-Puerta M, Patel NB, Huston BJ, Chavan S, Rosas-Ballina M, Gregersen PK, Czura CJ, Sloan RP, Sama AE, Tracey KJ. Cholinergic anti-inflammatory pathway activity and High Mobility Group Box-1 (HMGB1) serum levels in patients with rheumatoid arthritis. Mol Med 2007; 13(3-4):210-215.

[47] Sloan RP, McCreath H, Tracey KJ, Sidney S, Liu K, Seeman T. RR interval variability is inversely related to inflammatory markers: the CARDIA study. Mol Med 2007; 13(3-4):178-184.

[48] Marsland AL, Gianaros PJ, Prather AA, Jennings JR, Neumann SA, Manuck SB. Stimulated production of proinflammatory cytokines covaries inversely with heart rate variability. Psychosomatic Med 2007; 69(8):709-716.

[49] Kitagawa H, Takenouchi T, Azuma R, Wesnes KA, Kramer WG, Clody DE, Burnett AL. Safety, pharmacokinetics, and effects on cognitive function of multiple doses of GTS-21 in healthy, male volunteers. Neuropsychopharmacology 2003; 28(3):542-551.

[50] Pavlov VA, Tracey KJ. The cholinergic anti-inflammatory pathway. Brain Behav Immun 2005; 19(6):493-499.

[51] Gidron Y, Perry H, Glennie M. Does the vagus nerve inform the brain about preclinical tumours and modulate them? Lancet Oncol 2005; 6(4):245-248.

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