

Neuro-immune interactions via the cholinergic anti-inflammatory pathway

Margot Gallowitsch-Puerta^a, Valentin A. Pavlov^{a,b,*}

^a Laboratory of Biomedical Science, The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, USA

^b Center for Immunology and Inflammation, The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, USA

Received 1 November 2006; accepted 6 January 2007

Abstract

The overproduction of TNF and other cytokines is associated with the pathophysiology of numerous diseases. Controlling cytokine synthesis and release is critical for preventing unrestrained inflammation and maintaining health. Recent studies identified an efferent vagus nerve-based mechanism termed “the cholinergic anti-inflammatory pathway” that controls cytokine production and inflammation. Here we review current advances related to the role of this pathway in neuro-immune interactions that prevent excessive inflammation. Experimental evidence indicates that vagus nerve cholinergic anti-inflammatory signaling requires alpha7 nicotinic acetylcholine receptors expressed on non-neuronal cytokine-producing cells. Alpha7 nicotinic acetylcholine receptor agonists inhibit cytokine release and protect animals in a variety of experimental lethal inflammatory models. Knowledge related to the cholinergic anti-inflammatory pathway can be exploited in therapeutic approaches directed towards counteracting abnormal chronic and hyper-activated inflammatory responses.

© 2007 Elsevier Inc. All rights reserved.

Keywords: Cholinergic anti-inflammatory pathway; Vagus nerve; TNF; Inflammation; Acetylcholine; $\alpha 7$ nicotinic acetylcholine receptor; Muscarinic acetylcholine receptors

Introduction

Nervous system interaction with the immune system is vital for modulating innate immune responses and controlling inflammation (Tracey, 2002; Pavlov et al., 2003; Pavlov and Tracey, 2004; Czura and Tracey, 2005). Inflammation, a highly regulated response to infection and injury, has evolved as a beneficial component of the physiological defense systems of the host organism. Inflammation is critically mediated by tumor necrosis factor (TNF) and other pro- and anti-inflammatory cytokines, which are produced by activated macrophages and other innate immune cells (Tracey et al., 1986; Tracey, 2002). The production and release of cytokines are part of the advantageous response of the host innate immune system towards neutralizing the invading pathogen and promoting wound healing. These benefits are an asset to host survival; however, the innate immune

mechanisms underlying inflammatory responses must be extremely well balanced in order to prevent the deleterious effects of overproduction of TNF and other pro-inflammatory cytokines that can result in systemic inflammation and secondary tissue injury (Tracey, 2002). Abnormal systemic inflammation is a characteristic event associated with the pathology of rheumatoid arthritis, inflammatory bowel diseases, sepsis and other disorders (Tracey, 2002). Systemic inflammation can also be experimentally induced by administering lipopolysaccharide (LPS, endotoxin), an active major component of the outer membranes of Gram-negative bacteria and prototypical activator of innate immune responses. Recombinant human TNF (cachectin) elicits the same pathophysiological consequences that are caused by high dose endotoxin administration in rats including hypotension, metabolic acidosis, hemoconcentration and death occurring within hours after TNF administration (Tracey et al., 1986). Animals receiving neutralizing anti-TNF monoclonal antibody fragments prior to bacterial challenge are completely protected against shock, organ dysfunction, and death (Tracey et al., 1987). The discovery that administration of TNF, in doses similar to the levels produced by the host in response to endotoxin, evoked the symptoms caused by

* Corresponding author. Laboratory of Biomedical Science, The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, USA. Tel.: +1 516 562 2316; fax: +1 516 562 2356.

E-mail address: vpavlov@nshs.edu (V.A. Pavlov).

endotoxin administration was paramount. This scientific breakthrough identified TNF as a necessary and sufficient mediator of systemic inflammation. These findings indicated the possibility that anti-TNF strategies could be used in the treatment of life threatening diseases characterized by abnormally elevated TNF levels (Tracey et al., 1987). The overabundance of TNF and other pro-inflammatory cytokines may be lethal and conserved physiological mechanisms have evolved to counteract pro-inflammatory cytokine excess. Anti-inflammatory mechanisms include the release of glucocorticoids, anti-inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF- β), and soluble receptors which neutralize the activity of cytokines. Recently, a neural efferent vagus nerve-mediated mechanism that can suppress the overproduction of TNF and other pro-inflammatory cytokines was described (Borovikova et al., 2000). This pathway, termed the “cholinergic anti-inflammatory pathway” represents a physiological mechanism by which the nervous system interacts with the innate immune system to restrain systemic inflammatory responses.

The immunomodulatory and anti-inflammatory function of the efferent vagus nerve

The vagus nerve is the tenth cranial nerve and major constituent of the parasympathetic part of the autonomic nervous system. It contains sensory (afferent) and motor (efferent) fibers. The efferent vagus nerve arises in the brainstem medulla oblongata and innervates visceral organs. The vagus nerve is traditionally associated with the regulation of vital physiological functions including heart rate, bronchoconstriction, and gastrointestinal function through its principal neurotransmitter, acetylcholine as well as other neuronal and humoral substances (Pavlov and Tracey, 2004). Research in our laboratory and others has shown that the efferent vagus nerve inhibits pro-inflammatory cytokine production and systemic inflammation, thus identifying a non-classical immunoregulatory and anti-inflammatory function of the efferent vagus nerve (Borovikova et al., 2000; Wang et al., 2003; De Jonge et al., 2005; Huston et al., 2006; Ghia et al., 2006) (Fig. 1). Accumulating evidence indicates that this regulation requires interaction between efferent vagus nerve signaling and nicotinic acetylcholine receptors expressed on macrophages and other non-neuronal cytokine-producing cells that reside in organs of the reticuloendothelial system. These findings, together with the major neurotransmitter function of acetylcholine in pre- and post-ganglionic efferent vagal neurons as well as its anti-inflammatory role in vitro (Borovikova et al., 2000), have given rise to the concept of the efferent vagus nerve driven cholinergic anti-inflammatory pathway. Animals receiving direct electrical stimulation of the vagus nerve exhibit significantly reduced systemic levels of TNF and other pro-inflammatory cytokines during endotoxemia (Borovikova et al., 2000; Wang et al., 2003; Pavlov et al., 2006). Interestingly, vagus nerve stimulation does not change systemic anti-inflammatory cytokine levels (Borovikova et al., 2000). Pro-inflammatory cytokine levels are higher in animals subjected to vagotomy during endotoxemia

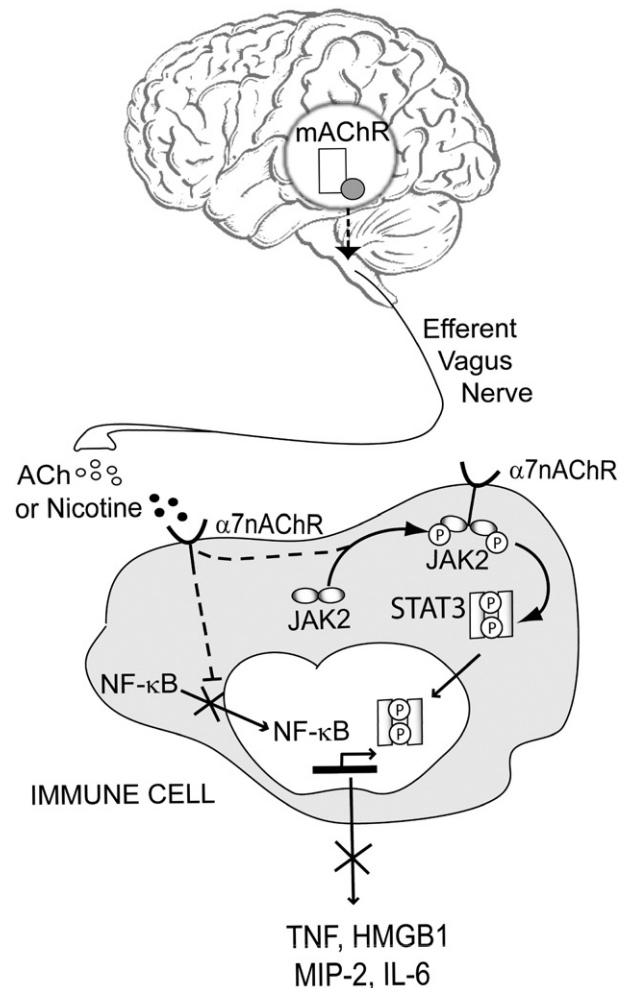


Fig. 1. Cholinergic mechanisms of modulating immune function and controlling inflammation (proposed mechanisms). Efferent vagus nerve cholinergic signaling inhibits TNF and other pro-inflammatory cytokine levels through $\alpha 7 n A C h R$ -mediated mechanisms. Experimental evidence indicates that activation of $\alpha 7 n A C h R$ on immune cells by nicotine can prevent NF- κ B nuclear translocation. $\alpha 7 n A C h R$ -mediated activation of the JAK2/STAT3 pathway has also been demonstrated in response to nicotine. These signaling pathways play a central role in transmitting vagal or nicotine-induced cholinergic anti-inflammatory signaling leading to inhibition of immune cell activation and suppression of TNF, HMGB1, macrophage inflammatory protein-2 (MIP-2) and IL-6 synthesis. Brain muscarinic acetylcholine receptor (mAChR) signaling mechanisms also modulate vagal immunoregulatory and anti-inflammatory output (see text for details).

(Borovikova et al., 2000) and intestinal inflammation (Ghia et al., 2006), which indicates a tonic inhibitory effect of the vagus nerve on pro-inflammatory cytokine production. Vagus nerve stimulation also attenuates the development of lethal shock in endotoxemic rats (Borovikova et al., 2000). Moreover, vagus nerve stimulation significantly inhibits serum, cardiac and hepatic TNF levels as well as attenuates the development of shock in animal models of ischemia–reperfusion as a result of aortic occlusion (Bernik et al., 2002) and hypovolemic hemorrhagic shock (Guarini et al., 2003). The development of acute colitis in mice also is dependent on the anti-inflammatory function of the vagus nerve (Ghia et al., 2006). These findings have indicated the profound beneficial effect of the efferent

vagus nerve in several experimental models of diseases as shown in Table 1.

Current knowledge indicates that the vagus nerve provides an important bi-directional communication circuit by which the brain modulates inflammation (Tracey, 2002; Pavlov et al., 2003). The presence of inflammation can be detected by the sensory (afferent) vagus nerve and communicated to the nucleus tractus solitarius in the brainstem medulla oblongata. Neural communication between this other brainstem nuclei and “higher” brain structures including the hypothalamus are associated with the generation of brain-derived anti-inflammatory output through the efferent vagus nerve-mediated cholinergic anti-inflammatory pathway (Tracey, 2002; Pavlov et al., 2003; Pavlov and Tracey, 2005). Knowledge related to this vagus nerve dominated “inflammatory reflex” and the mechanisms of its regulation contribute to a more detailed understanding of the neuro-immune interactions which regulate innate immune responses and inflammation. A recent study demonstrates this reflex through a dietary fat-induced vago-vagal mechanism that controls inflammation (Luyer et al., 2005). Dietary fat intake causes the release of cholecystokinin (CCK), activation of CCK receptors and consequent stimulation of afferent and efferent vagus nerve activity which results in the inhibition of pro-inflammatory cytokines in rats during hemorrhagic shock (Luyer et al., 2005). This study also sheds new light on the role that nutrition may have in the cholinergic anti-inflammatory pathway (Luyer et al., 2005). In addition to the vago-vagal neural inflammatory reflex, humoral pathways also are stimulated, and activation of the hypothalamo–pituitary–adrenal axis results in the release of glucocorticoids producing an anti-inflammatory signal. Activation of the sympathetic division of the autonomic nervous system may also be induced during inflammation resulting in the release of epinephrine (adrenaline) and norepinephrine (noradrenaline), which are involved in the complex receptor-dependent regulation of inflammatory responses (Pavlov and Tracey, 2004). Thus, the

host organism mobilizes neural and neurohumoral mechanisms to control inflammation during an immune challenge.

Mechanisms of anti-inflammatory cholinergic signaling

Research in our laboratory utilizing antisense and knockout approaches identified the critical importance of the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR) in mediating cholinergic anti-inflammatory signaling (Wang et al., 2003). Experiments with $\alpha 7$ nAChR knockout mice revealed that in the absence of the $\alpha 7$ nAChR, vagus nerve stimulation was ineffective at preventing TNF release indicating that the $\alpha 7$ nAChR is essential for the effectiveness of the cholinergic anti-inflammatory pathway (Wang et al., 2003). Recently, a functional connection between the vagus nerve anti-inflammatory activity and the spleen was identified (Huston et al., 2006). The spleen was shown to be a major producer of TNF during endotoxemia, and vagus nerve stimulation significantly suppressed splenic TNF through an $\alpha 7$ nAChR-mediated mechanism (Huston et al., 2006). Further studies are needed to elucidate the mechanisms involved and the importance of this regulation under different inflammatory conditions.

The $\alpha 7$ nAChR is expressed on non-neuronal cells including macrophages, endothelial cells, dendritic cells, keratinocytes and lymphocytes (Grando et al., 2003; Saeed et al., 2005; Kawashima et al., 2007). In addition, these cells express other markers of the cholinergic system, including acetylcholine release, a variety of other nicotinic and muscarinic acetylcholine receptors, and the enzymes choline acetyltransferase and acetylcholinesterase, thus forming a non-neuronal cholinergic system (Grando et al., 2003; Kawashima et al., 2007). While macrophages have been identified as the major source of TNF during endotoxemia, dendritic cells, endothelial cells and lymphocytes also synthesize and release pro-inflammatory cytokines and are substantial contributors to the innate immune activation underlying inflammatory responses. Macrophages also have a prominent role in mediating intestinal inflammation, and a recent study has identified these cells as the main target of the anti-inflammatory function of the vagus nerve in a murine model of inflammatory bowel disease (Ghia et al., 2006).

Although muscarinic acetylcholine receptors are expressed on macrophages and other cytokine-producing cells they do not seem to play a critical role in transmitting vagal anti-inflammatory output during endotoxemia (Pavlov et al., 2006). Administration of atropine methyl nitrate, which blocks peripheral muscarinic receptors including those expressed on cytokine-producing cells, does not interrupt the TNF suppressing effect of vagus nerve stimulation in endotoxemic rats (Pavlov et al., 2006). These findings highlight a major difference between classical vagal cholinergic regulation of physiological functions such as heart rate, etc. that are predominantly mediated by muscarinic receptors and the immunomodulatory function of the vagus nerve, mediated by the $\alpha 7$ nAChR. Moreover, we have recently demonstrated a role for muscarinic receptors in the central nervous system (CNS) in inhibiting systemic inflammation in endotoxemic rats (Pavlov et al., 2006). We have shown that activation of muscarinic cholinergic transmission in the CNS by muscarinic receptor ligands lowers serum TNF levels

Table 1
Anti-inflammatory role of the vagus nerve and/or nicotine in experimental models of diseases^a

Disease	Vagus Nerve Stimulation/ Vagotomy	Nicotine
Endotoxemic shock	Borovikova et al. (2000) Wang et al. (2003) Wang et al. (2004) Pavlov et al. (2006) Huston et al. (2006)	Wang et al. (2004)
Sepsis		Wang et al. (2004) Huston et al. (2006)
Hemorrhagic shock	Guarini et al. (2003, 2004) Luyer et al. (2005)	
Ischemia–reperfusion	Bernik et al. (2002)	
Subcutaneous inflammation	Saeed et al. (2005)	Saeed et al. (2005)
Postoperative ileus	De Jonge et al. (2005)	De Jonge et al. (2005)
Inflammatory bowel disease	Ghia et al. (2006)	Ghia et al. (2006)

^a Please note that due the required limited reference number we could not refer to all studies related to the anti-inflammatory function of the vagus nerve and/or nicotine.

(Pavlov et al., 2006). We have also shown that central muscarinic cholinergic activation results in higher efferent vagus nerve activity (as demonstrated by an increase in the high frequency power component of heart rate variability), thus indicating a role for the efferent vagus nerve in conveying the central cholinergic signal to the periphery, which leads to inhibition of systemic TNF levels. Our study, together with observations that muscarinic receptors in the CNS are involved in controlling the vagus nerve anti-inflammatory function during hemorrhagic shock in rats (Guarini et al., 2004), indicates a role for central muscarinic receptor mechanisms in controlling the cholinergic anti-inflammatory pathway in rats. The central (brain) neuronal circuits underlying this regulation remain enigmatic.

The discovery of the critical role for the $\alpha 7$ nAChR in mediating cholinergic anti-inflammatory signaling led to the utilization of nicotine and other $\alpha 7$ nAChR agonists in mechanistic studies. Nicotine has been shown to inhibit nuclear factor kappa B (NF- κ B) translocation to the nucleus in endotoxin-stimulated RAW 264.7 macrophages (Wang et al., 2004) (Fig. 1). NF- κ B is a key transcription factor for the synthesis of TNF and other cytokines and preventing its nuclear translocation in response to endotoxin and other immunogenic stimuli is critical for decreasing pro-inflammatory cytokine production. Vagus nerve stimulation causes nicotinic receptor-dependent suppression of hepatic NF- κ B activation during hemorrhagic shock (Guarini et al., 2003). These data combined with the finding of lower hepatic TNF mRNA and serum protein levels as a result of vagus nerve stimulation provide additional evidence that cholinergic anti-inflammatory signaling is associated with suppression of NF- κ B activation in vivo (Guarini et al., 2003). It has been demonstrated that nicotine inhibits resident peritoneal macrophage activation ex vivo, attenuating pro-inflammatory cytokine release through $\alpha 7$ nAChR-mediated activation of the janus kinase (JAK) /signal transducer and activator of transcription (STAT) pathway (De Jonge et al., 2005). Binding of nicotine to the $\alpha 7$ nAChR triggers activation (phosphorylation) of JAK2 and subsequent phosphorylation of effectors such as STAT3. Phosphorylated STAT3 translocates to the nucleus and is correlated downstream with decreased levels of TNF, macrophage inflammatory protein 2 (MIP-2), and interleukin 6 (IL-6) (De Jonge et al., 2005; Gallowitsch-Puerta and Tracey, 2005) (Fig. 1). The importance of cholinergic anti-inflammatory signaling through the JAK/STAT pathway also is supported by the finding that the anti-inflammatory function of vagus nerve stimulation is abolished in STAT3 deficient mice (De Jonge et al., 2005). The involvement of other alternative intracellular pathways in mediating cholinergic anti-inflammatory signaling remains to be evaluated; although some of these mechanisms such as calcium signaling have been extensively studied in neurons, their mediating role in macrophages and other non-excitable cells is still enigmatic.

In addition to inhibiting TNF and other “early” pro-inflammatory cytokines, $\alpha 7$ nAChR-dependent cholinergic signaling also is implicated in suppressing the release of high mobility group box 1 (HMGB1), a “late” cytokine mediator of systemic inflammation (Wang et al., 2004). Studies have shown that HMGB1 plays important functions in mediating the pathology

of experimental severe sepsis and other inflammatory disorders (Wang et al., 1999; Tracey, 2005). Analysis of patients with sepsis and cerebral and myocardial ischemia has shown elevated systemic levels of HMGB1 (Wang et al., 1999; Goldstein et al., 2006), indicating the potential for this protein as a therapeutic target. In fact anti-HMGB1 antibodies have been shown to improve survival in experimental models of severe sepsis (Tracey, 2005). Treatment with nicotine results in lower systemic levels of HMGB1 in endotoxemic and septic mice and significantly increases survival rates when compared with vehicle administered controls (Wang et al., 2004).

In addition to the vagus nerve, the anti-inflammatory function of nicotine has been explored in various experimental models of diseases as summarized in Table 1. Future studies will contribute to validating the efficacy of these approaches in the clinical management of inflammatory diseases.

Conclusion

The cholinergic anti-inflammatory pathway is a physiological neuro-immune mechanism that regulates innate immune function and controls inflammation. Current knowledge indicates that the functional activity of this pathway can be modulated through its neuronal (efferent vagal neurons and higher brain structures) and non-neuronal ($\alpha 7$ nAChR on cytokine-producing cells) cholinergic components. Future studies on the neuro-immune interactions through the cholinergic anti-inflammatory pathway in rodents and humans will contribute to unraveling the immunoregulatory mechanisms and therapeutic potential of this pathway.

Acknowledgements

This work was supported by the Feinstein Institute for Medical Research Reward Program, North Shore-Long Island Jewish GCRC (General Clinical Research Center), MO1 RR018535, and the NIGMS (National Institute of General Medical Sciences). We would like to thank Kevin J. Tracey and William R. Parrish for critically reading this manuscript. We apologize to those authors whose work could not be cited because of the format (reference limitations) of this article.

References

- Bernik, T.R., Friedman, S.G., Ochani, M., DiRaimo, R., Susarla, S., Czura, C.J., Tracey, K.J., 2002. Cholinergic antiinflammatory pathway inhibition of tumor necrosis factor during ischemia reperfusion. *Journal of Vascular Surgery* 36 (6), 1231–1236.
- Borovikova, L.V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G.I., Watkins, L.R., Wang, H., Abumrad, N., Eaton, J.W., Tracey, K.J., 2000. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405 (6785), 458–462.
- Czura, C.J., Tracey, K.J., 2005. Autonomic neural regulation of immunity. *Journal of Internal Medicine* 257, 156–166.
- De Jonge, W.J., van der Zanden, E.P., The, F.O., Bijlsma, M.F., van Westerloo, D.J., Bennis, R.J., Berthoud, H.R., Uematsu, S., Akira, S., van den Wijngaard, R.M., Boeckxstaens, G.E., 2005. Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2–Stat3 signaling pathway. *Nature Immunology* 6, 844–851.

- Gallowitsch-Puerta, M., Tracey, K.J., 2005. Immunologic role of the cholinergic anti-inflammatory pathway and the nicotinic acetylcholine $\alpha 7$ receptor. *Annals of the New York Academy of Science* 1062, 1–11.
- Ghia, J.E., Blennerhassett, P., Kumar-Ondiveeran, H., Verdu, E.F., Collins, S.M., 2006. The vagus nerve: a tonic inhibitory influence associated with inflammatory bowel disease in a murine model. *Gastroenterology* 131 (4), 1122–1130.
- Goldstein, R.S., Gallowitsch-Puerta, M., Yang, L., Rosas-Ballina, M., Huston, J.M., Czura, C.J., Lee, D.C., Ward, M.F., Bruchfeld, A.N., Wang, H., Lesser, M.L., Church, A.L., Litroff, A.H., Sama, A.E., Tracey, K.J., 2006. Elevated high-mobility group box 1 levels in patients with cerebral and myocardial ischemia. *Shock* 25 (6), 571–574.
- Grando, S.A., Kawashima, K., Wessler, I., 2003. Introduction: the non-neuronal cholinergic system in humans. *Life Sciences* 72 (18–19), 2009–2012.
- Guarini, S., Altavilla, D., Cainazzo, M.M., Giuliani, D., Bigiani, A., Marini, H., Squadrito, G., Minutoli, L., Bertolini, A., Marini, R., Adamo, E.B., Venuti, F.S., Squadrito, F., 2003. Efferent vagal fibre stimulation blunts nuclear factor-kappaB activation and protects against hypovolemic hemorrhagic shock. *Circulation* 107 (8), 1189–1194.
- Guarini, S., Cainazzo, M.M., Giuliani, D., Mioni, C., Altavilla, D., Marini, H., Bigiani, A., Ghiaroni, V., Passaniti, M., Leone, S., Bazzani, C., Caputi, A.P., Squadrito, F., Bertolini, A., 2004. Adrenocorticotropin reverses hemorrhagic shock in anesthetized rats through the rapid activation of a vagal anti-inflammatory pathway. *Cardiovascular Research* 63 (2), 357–365.
- Huston, J.M., Ochani, M., Rosas-Ballina, M., Liao, H., Ochani, K., Pavlov, V.A., Gallowitsch-Puerta, M., Ashok, M., Czura, C.J., Foxwell, B., Tracey, K.J., Ulloa, L., 2006. Splenectomy inactivates the cholinergic anti-inflammatory pathway during lethal endotoxemia and polymicrobial sepsis. *Journal of Experimental Medicine* 203 (7), 1623–1628.
- Kawashima, K., Yoshikawa, K., Fujii, Y., Moriwaki, Y., 2007. Expression and function of genes encoding cholinergic components in murine immune cells. *Life Sciences* 80, 2314–2319.
- Luyer, M.D., Greve, J.W., Hadfoune, M., Jacobs, J.A., Dejong, C.H., Buurman, W.A., 2005. Nutritional stimulation of cholecystokinin receptors inhibits inflammation via the vagus nerve. *Journal of Experimental Medicine* 202 (8), 1023–1029.
- Pavlov, V.A., Tracey, K.J., 2004. Neural regulators of innate immune responses and inflammation. *Cellular and Molecular Life Sciences* 61, 2322–2331.
- Pavlov, V.A., Tracey, K.J., 2005. The cholinergic anti-inflammatory pathway. *Brain Behavior and Immunity* 19 (6), 493–499.
- Pavlov, V.A., Wang, H., Czura, C.J., Friedman, S.G., Tracey, K.J., 2003. The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. *Molecular Medicine* 9, 125–134.
- Pavlov, V.A., Ochani, M., Gallowitsch-Puerta, M., Ochani, K., Huston, J.M., Czura, C.J., Al-Abed, Y., Tracey, K.J., 2006. Central muscarinic cholinergic regulation of the systemic inflammatory response during endotoxemia. *Proceedings of the National Academy of Sciences of the United States of America* 103 (13), 5219–5223.
- Saeed, R.W., Varma, S., Peng-Nemeroff, T., Sherry, B., Balakhaneh, D., Huston, J., Tracey, K.J., Al-Abed, Y., Metz, C.N., 2005. Cholinergic stimulation blocks endothelial cell activation and leukocyte recruitment during inflammation. *Journal of Experimental Medicine* 201 (7), 1113–1123.
- Tracey, K.J., 2002. The inflammatory reflex. *Nature* 420, 853–859.
- Tracey, K.J., 2005. *Fatal Sequence: The Killer Within*. Dana Press, Washington, DC, pp. 128–204.
- Tracey, K.J., Beutler, B., Lowry, S.F., Merryweather, J., Wolpe, S., Milsark, I.W., Hariri, R.J., Fahey III, T.J., Zentella, A., Albert, J.D., et al., 1986. Shock and tissue injury induced by recombinant human cachectin. *Science* 234 (4775), 470–474.
- Tracey, K.J., Fong, Y., Hesse, D.G., Manogue, K.R., Lee, A.T., Kuo, G.C., Lowry, S.F., Cerami, A., 1987. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature* 330 (6149), 662–664.
- Wang, H., Bloom, O., Zhang, M., Vishnubhakat, J.M., Ombrellino, M., Che, J., Frazier, A., Yang, H., Ivanova, S., Borovikova, L., Manogue, K.R., Faist, E., Abraham, E., Andersson, J., Andersson, U., Molina, P.E., Abumrad, N.N., Sama, A., Tracey, K.J., 1999. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 285 (5425), 248–251.
- Wang, H., Yu, M., Ochani, M., Amella, C.A., Tanovic, M., Susarla, S., Li, J.H., Wang, H., Yang, H., Ulloa, L., Al-Abed, Y., Czura, C.J., Tracey, K.J., 2003. Nicotinic acetylcholine receptor $\alpha 7$ subunit is an essential regulator of inflammation. *Nature* 421 (6921), 384–388.
- Wang, H., Liao, H., Ochani, M., Justiniani, M., Lin, X., Yang, L., Al-Abed, Y., Wang, H., Metz, C., Miller, E.J., Tracey, K.J., Ulloa, L., 2004. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nature Medicine* 10 (11), 1216–1221.