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Neural Reflex Control of Inflammation During Sepsis Syndromes

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1. Introduction

"Healthy organs behave as 'biological oscillators', which couple to one another, and this orderly coupling is maintained through a communication network, including neural, humoral, and cytokine components" (Godin & Buchman, 1996). The nervous system –acting through the autonomic nervous system (ANS)– coordinates the fine-tuning of cardiorespiratory interplay, to maintain an appropriate oxygen delivery to the tissues (Abboud & Thames, 1983; Eyzaguirre *et al.*, 1983). Autonomic (sympathetic-parasympathetic) balance is maintained by several reflex arcs, like arterial baroreflexes (Kirchheim, 1976), central chemoreflexes, peripheral arterial chemoreflexes, and pulmonary stretch reflexes (Liljestrand, 1958). These reflexes represent the major components of blood pressure and breathing regulation. Therefore, the interactions among these reflexes are of special clinical interest, since the overactivity of a single reflex, occurring pathophysiologically in several disorders, can lead to the suppression of opposite reflex responses (Schmidt *et al.*, 2001).

Sepsis syndromes (SS), which include systemic inflammatory response syndrome (SIRS) and its consequences, severe sepsis and septic shock, involve many pathological processes like systemic inflammation, coagulopathies, hemodynamic abnormalities, and multiple organ dysfunction syndrome (MODS) (Riedemann *et al.*, 2003). The progression of MODS associated to systemic inflammation is mainly due to an uncontrolled release of pro-inflammatory mediators, which damage parenchymatous organs. Additionally, sepsis activates and/or depress numerous other systems within the body, including neural, hormonal, and metabolic pathways (Carre & Singer, 2008; Singer *et al.*, 2004). Thus, systemic inflammation would initiates disruption of communication and uncoupling, and subsequent MODS would reflects the progressive uncoupling of 'biological oscillators' that can become irremediable.

Increasing evidences here summarized shown that a particular neural reflection, the carotid body chemoreflexes, not only serves as a chemoreceptor for respiratory reflex responses, as traditionally accepted, but also as a sensor for the immune status, as modulator of autonomic balance tending to coordinate cardiorespiratory interplay, devoted to maintain oxygen homeostasis in different pathologies, and as a protective factor during sepsis and MODS.

2. Sepsis syndromes prevalence and current therapies

Sepsis is defined as "the systemic inflammatory response that occurs during infection" (Bone *et al.*, 1992). It involves the evidence of infection and two or more of the following conditions: fever or hypothermia, tachycardia, tachypnea or a respiratory frequency resulting in an arterial PCO_2 below 32 mm Hg, and altered white blood cells count; *severe sepsis*, sepsis associated with organ dysfunction, hypoperfusion or hypotension including lactic acidosis, oliguria, or acute alteration in mental state; *septic shock*, sepsis-induced hypotension despite adequate fluid resuscitation, and sustained perfusion abnormalities; and *multiple organ dysfunction syndrome (MODS)*, by the presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention (Riedemann *et al.*, 2003).

Significant demographic variation exists in the risk of developing sepsis. For example, from the standpoint of gender, the incidence of sepsis is higher in men, and the mean age at which men develop sepsis is younger. Case fatality rates also increase with age (Martin *et al.*, 2006). The overall burden of severe sepsis is also increasing, in terms of both the number of patients who develop the syndrome and the extent and intensity of care that they require (Angus *et al.*, 2001). Sepsis also poses a significant burden of disease in pediatric patients, where the incidence is highest in infants, mainly in children younger than one year of age (Watson & Carcillo, 2005). Maternal sepsis and neonatal sepsis are of particular concern. Maternal sepsis is responsible for at least 75,000 deaths annually, disproportionately affecting low-income countries (van Dillen *et al.*, 2010). In the United States, studies of neonatal sepsis have documented rates as high as 170 cases per 1000 live births (Thaver & Zaidi, 2009). The average costs per case are US$22,100. Costs are higher in infants, non-survivors, intensive care unit patients, surgical patients, and patients with more organ failure. The incidence was projected to increase by 1.5% per annum (Angus *et al.*, 2001). The international costs associated with sepsis and its management are reviewed in Chalupka & Talmor (2012).

Instead of many efforts and significant advances in maintaining therapies, SS and MODS, are the main cause of death between critical care patients (Martin *et al.*, 2003). Increased morbi-mortality associated to SS is due to the absence of a really effective therapy (Riedemann *et al.*, 2003). Thus, the knowledge of molecular mechanisms and pathophysiology of sepsis help us to improve current therapies (for a Review see Barochia *et al.*, 2010) and to identify new pharmacological therapeutic targets.

Treatments of sepsis and septic shock involves antibiotic administration, intravenous fluids (crystalloids or colloids), vasopressors and/or inotropes (adrenergic agents), packed red blood cells (PRBC) transfusions, and corticosteroids (Barochia *et al.*, 2010). Sepsis care bundles increase patients' survival. Numerous studies have demonstrated improved outcomes in life-threatening infections with early administration of appropriate antibiotics. Hemodynamic support with fluids and vasopressors is as important as antibiotic in reducing mortality (Natanson *et al.*, 1990), but there are great differences among different patient populations. A considerable variation in the ranges of central venous pressure and mean arterial pressure prompted physicians to suggest that "*the usage should be individualized to different patients, based on their own underlying medical conditions*" (Perel, 2008).

Administration of PRBC decreases inotropes use (Nguyen *et al.*, 2007), but the efficacy of administration in patients with sepsis is unclear. The usage of low-dose corticosteroids is variable between patient populations. However, as questions persist regarding the risk and benefits of these therapies for sepsis, they continue to undergo investigation (Misset *et al.*, 2010). Although the use of these agents may be beneficial for some septic patients, the Surviving Sepsis Campaign guidelines (Dellinger *et al.*, 2004) gave a weak recommendation for use these therapies, even the inclusion of some patients, until the knowledge of individual components that could modify the expected results. It is clear that the course of sepsis and therapies outcomes depend largely from host predisposition factors and response.

The serial evaluation of the SOFA score helps to predict outcome in critically ill patients. SOFA score can help assess organ dysfunction or failure over time and are useful to evaluate morbidity and mortality, by evaluating respiratory, coagulation, liver, cardiovascular, central nervous system (CNS), and renal variables (Peres *et al.*, 2002). However, in spite of SOFA score assessment, "*It is more important to know what sort of person this disease has, than what sort of disease this person has*" (William Osler, 1849-1919).

3. Pathophysiology of sepsis and multiple organ dysfunction syndrome

As it was mentioned, the progression of MODS is due to an uncontrolled release of pro-inflammatory mediators, which damage parenchymatous organs. However, it is still unknown why sepsis progresses to MODS in only certain individuals or what the exact pathway is that leads to this. But, it is clear that if the inflammatory process becomes self-sustained and progressive, MOD results. In addition, because of marked hypotension and tissue hypoperfusion, oxygen delivery fails to meet tissue oxygen demands, which results in a compensatory increase in oxygen extraction. If the imbalance between oxygen delivery and consumption is not corrected, tissue 'dysoxia' progress to an anaerobic metabolism and lactate production (Nguyen *et al.*, 2004). Persistent serum lactate elevation is an important marker of decreased tissue perfusion –even in the absence of arterial hypotension (Howell *et al.*, 2007)–, and is strongly associated with mortality rate in critically ill patients (Meregalli *et al.*, 2004). Thus, during sepsis an extraordinarily complex and intricate cascade of inflammatory mediators, extra- and intra-cellular signaling pathways are activated,

resulting in microvascular dysregulation and/or mitochondrial dysfunction ('cytopathic hypoxia') (Crouser, 2004), which culminate in MODS and death.

To avoid tissue dysoxia, early in the course of sepsis, cardiac output (CO) rises to maintain blood pressure and organ perfusion in the face of reduced peripheral vascular resistance ('hyperdynamic sepsis'). As sepsis progresses, CO is frequently reduced ('hypodynamic sepsis'), which has a poor prognosis. Cardiac dysfunction *per se* is apparent in up to 44% of critically ill septic patients, with the etiological agents suspected to be circulating depressant factors (Singh & Evans, 2006). Elevated cardiac biomarkers (*e.g.*, Troponin I (ver Elst *et al.*, 2000; Yucel *et al.*, 2008)) in conjunction with electrocardiographic (ECG) changes are valuable in the diagnostic of sepsis and in the assessment of progression. Raised Troponin I levels in patients with sepsis result from various mechanisms, including hypoperfusion or direct extension of infection to cardiac tissue. Electrocardiographic changes in sepsis are not as well described. Some of them include loss of QRS amplitude, increase in corrected QT (QTc) interval, bundle branch blocks, and development of narrowed QRS intervals with deformed, positively deflected J waves (Martinez *et al.*, 2009).

Contradictory evidences from animal studies suggest that such hypoperfusion does not invariably lead to heart dysfunction and death. But, our preliminary results (unpublished data) reveal many other ECG and vectorcardiographic changes in rats injected intraperitoneally (i.p.) with 15 mg/kg lipopolysaccharide (LPS), which are strongly associated with cardiac dysfunction and, almost certainly, left vetricular hypoperfusion and ischemia. Briefly, LPS administration decreases RR interval (RRI) and R amplitude. Also, sepsis increases QTc interval and ST height. Strikingly, when both carotid/sinus nerves are sectioned (bilateral carotid neurotomy (BCN) prior to LPS administration, the changes in the parameters mentioned above are greater than control condition (with intact carotid chemo- and baro-sensory innervations). In addition, BCN decreases QRS duration, increases JT interval and T amplitude. On the other hand, the cardiac vector is significantly decreased (from *ca.* 65º to *ca.* 15º)

As it was mentioned, the major task toil of autonomic nervous system (ANS) is the fine-tuning of the cardiorespiratory interplay, in order to maintain an appropriate oxygen delivery to the tissues. However, the neural regulation of cardiorespiratory function and the role-played by peripheral reflexes during sepsis, in which organ communications networks are disrupted, is poorly understood. In addition to plasma or urinary levels of neurotransmitters or their metabolites, there are three methods to evaluate autonomic function: a) analysis of heart rate variability (HRV); b) baroreflex sensitivity (BRS); and c) cardiac chemoreflex sensitivity (CCRS).

The analysis of HRV gives a clear idea about the neural (autonomic) control of cardiorespiratory function and interaction. Decreased HRV is consistent with the pathogenesis of MODS, which involves the physiological uncoupling of vital organ systems. In fact, HRV decreases in response to human endotoxemia (Godin *et al.*, 1996; Rassias *et al.*, 2005), and is a good index of cardiac mortality (Schmidt *et al.*, 2001). Moreover, patients with sepsis (Barnaby *et al.*, 2002) and MODS (Korach *et al.*, 2001;

Schmidt *et al.*, 2005) have an impaired sympatho-vagal balance. In fact, some evidences describe a sustained sympatho-excitation during sepsis, which accompanies the fall in blood pressure. Baroreceptors and chemoreceptors denervation accelerated the fall in mean blood pressure and increases sympathetic tone (Vayssettes-Courchay *et al.*, 2005). Thus, under altered baro- and chemo-reflexes pathways, the sympathetic output from the *medulla* appears to play a key role in the correlation between heart rate and sympathetic nerve activity. On the other hand, decreased parasympathetic tone is a good predictor of risk of death in patients with sepsis (Chen *et al.*, 2008). Altogether, these data suggest that reflex arcs involved in maintaining the autonomic balance are altered during sepsis.

Vayssettes-Courchay *et al.* (2005) shown that baro- and chemo-reflexes are not inhibited during sepsis, and they give them a minor importance in the sympathetic activation and in the blood pressure modifications. Nevertheless, recently we described the first functional evidence of chemoreceptors inflammation and dysfunction during sepsis. In cats, local or systemic administration of LPS induces a significant reduction in chemoreceptor activity, ventilatory chemoreflexes, and ventilator chemosensory drive (Fernandez *et al.*, 2008). In fact, LPS-induced tachypnea is prevented by prior bilateral carotid neurotomy.

Our results (unpublished data) shown that the i.p. administration of 15 mg/kg LPS to rats, decreases HRV and increases sympathetic tone, assessed by HRV frequency bands and low frequency/high frequency (LF/HF) quotient. Bilateral carotid neurotomy previous to LPS administration evokes a greater decrease in HRV and increase in LF/HF ratio than animals with intact carotid/sinus nerves. As it was mentioned, both decreased HRV and increased sympathetic tone are good markers of morbi-mortality. In fact, BCN prior to LPS administration increases the relative risk of death (Table 1). In addition, rats submitted to peripheral chemodenervation prior to the intravenous (i.v.) administration of high doses of LPS, show a smaller survival time (Tang *et al.*, 1998).

	SHAM		BCN	
	saline	LPS	saline	LPS
Relative Risk (RR) (IC 95%)	1 (n=8)	1.2 (0.9 – 1.6) (n=12)	1.3 (0.9 – 1.8) (n=9)	2.6 (1.5 – 4.5)[a] (n=21)
Plasma Cortisol (ng/mL) (Mean ± SD)	536.5 ± 383.3 (n=7)	1552.0 ± 940.5[b] (n=7)	637.5 ± 397.0 (n=6)	321.5 ± 153.2[c] (n=6)

[a], p=0.0033 vs. SHAM-saline. Fisher's exact test

[b], p<0.05 vs. SHAM-saline. Kruskal-Wallis ANOVA, Dunn's post test.

[c], p<0.01 vs. SHAM-LPS. Kruskal-Wallis ANOVA, Dunn's post test.

Table 1. Summary of observations in rats submitted to bilateral carotid neurotomy (BCN) or simulated surgery (SHAM) prior to the i.p. administration of 15 mg/kg LPS (LPS) or vehicle (saline). The data were assessed 90-min after LPS or vehicle administration. Table prepared from part of the data presented in Reyes *et al.*, 2012 (In press. Adv Exp Med Biol)

Baroreflex sensitivity describes ANS capacity to increase vagal activity and to decrease sympathetic activity after a sudden increase in blood pressure. Baroreflex activation counteract sympathetic activation (Somers *et al.*, 1991). BRS is altered in rats treated with a lethal dose of LPS (Shen *et al.*, 2004). Rougaush *et al.* reported an increased BRS after bacterial sub-pyrogenic dose of endotoxin. The change in sensitivity may underlie necessary adjustments to altered blood flow distribution after LPS administration (Rogausch *et al.*, 2000). However, Schmidt *et al.* reported a marked decrease in BRS during MODS (Schmidt *et al.*, 2005). Thus, there is no consensus about the role played by arterial baroreceptors during sepsis. Classically, the stimulation of peripheral chemoreceptors evokes respiratory and cardiovascular effects and a sympatho-excitatory response (Alanis *et al.*, 1968; Montarolo *et al.*, 1976). Cardiac chemoreflex sensitivity (CCRS) allow us to estimate the sympathetic influence upon cardiorespiratory responses (Schmidt *et al.*, 1999). Hyperoxia decreases autonomic function –*i.e.*, decreased CCRS– and increases BRS. CCRS gives an important component of the cardiorespiratory interactions in patients with MODS. Severity of illness is the more pronounced determinant of impaired CCRS (Schmidt *et al.*, 2004). Recently, Schueller *et al.* described a reduced CCRS in critical ill patients (sepsis or cardiogenic shock). Moreover, there is a close negative correlation between the CCRS and the SOFA-score (Schueller *et al.*, 2008).

In summary, there is consensus that uncoupling of the autonomic, respiratory and cardiovascular systems occurs over both short- and long-range time scales during sepsis and MODS. However, the origin from these altered reflex arcs is not well described.

4. Inflammatory mediators during sepsis

The development of sequential organ failure in critically ill patients with sepsis is strongly predictive of mortality. However, the mechanisms involved in the dynamic interaction between different organ systems are dictated by the intricate interplay of homodynamic, oxygen transport, and metabolic disturbances. Genetic predisposition is almost certainly relevant in upregulating the expression of inflammatory mediators [*e.g.*, tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, high mobility group box (HMGB) 1], thereby influencing adversely the anti-/pro-inflammatory balance.

Mammals are continuously exposed to different pathogens, like Gram-negative bacteria and/or its components, such as LPS (endotoxin). LPS exerts many different biological effects. While low-doses could be beneficial, by inducing immunostimulation and by increasing resistance to infection (Schletter *et al.*, 1995), larger-doses of LPS in plasma evoke many pathophysiological reactions, like fever, leucopenia, tachycardia, tachypnea, hypotension, disseminated intravascular coagulation, MODS, and death (Patel *et al.*, 2003; Hotchkiss & Karl, 2003; Pinsky, 2004). The systemic inflammatory response induced by LPS is due to host cells stimulation (monocytes/macrophages, endothelial, and polymorphonuclear cells) to produce and release endogenous mediators like reactive oxygen species (ROS) and pro-inflammatory cytokines (Schletter *et al.*, 1995). Inflammatory mediators and ROS are believed to disrupt communication pathways between organs, which precedes organ

failure. Indeed, endothelial dysfunction has been proposed as a common pathway for organ dysfunction in sepsis (Simon & Fernandez, 2009). During systemic inflammation, many physiological functions of endothelial cells are disrupted, contributing to multiple organ failure (Volk & Kox, 2000).

During the last decade, there has been a rapid progress in understanding innate immune response to pathogens or their component. The early concept supposed a nonspecific recognition. But, the discovery of Toll-like receptors (TLRs) showed that recognition by the innate immune system is specific (Akira *et al.*, 2001). TLR-4 is identified as the long-sought receptor that respond to bacterial LPS (Akira *et al.*, 2006). TLR4 forms a complex with MD-2 on the cell surface. Additional proteins such as the soluble plasma protein LPS-binding protein (LBP) and either soluble or membrane-anchored CD14 are also involved in LPS binding (Akashi-Takamura & Miyake, 2008). LPS transfer to the LPS-binding receptor (TLR-4/MD-2) (da Silva *et al.*, 2001), activates nuclear factor-κB (NF-κB), a transcription factor involved in the synthesis and release of immune system-related cytotoxic factors, by stimulation of pro-inflammatory and immunoregulatory molecules synthesis in mononuclear cells (monocytes /macrophages and neutrophils), like IL-1, IL-6, TNF-α, IL-10, and transforming growth factor (TGF)-β (Medvedev *et al.*, 2000;Sanlioglu *et al.*, 2001). Increased plasma levels of TNF-α, IL-1 and IL -6, γ-interferon (IFN-γ), and TGF-β are present in patients with different pathological conditions (Schletter *et al.*, 1995), but a particular cytokine, TNF-α, seems to play a pivotal role during sepsis and MODS (Tracey *et al.*, 1986).

Tumor necrosis factor-α has been implicated as an important mediator of the lethal effect of endotoxin. Several publications have shown that by reducing the activity or the expression of TNF-α significantly decrease the endotoxin-induced damages. The amount of TNF-α in serum can be associated with the degree of tissue damage because of the stagnant blood capillary (Yang *et al.*, 2007). TNF-α is a well-known cytotoxic cytokine for certain tissue cells. In fact, plasma levels of several biophysical damage indicators are increased during sepsis, like liver alanine aminotransferase, aspartate aminotransferase, and bilirrubin; heart and other possible organ (such as muscle) lactic dehydrogenase and creatine phosphokinase; ureic nitrogen (BUN, renal function); and pancreatic alkaline phosphatase and amylase.

5. Reflex control of inflammation: Part I – Brain-to-immune communication

Inflammation is a localized protective response to infection or injury. It evokes many different effects upon the organisms tending to solve the inflammatory focus, like humoral factors which increase the blood flow or attract specific immune cells (Libert, 2003). As it was mentioned above, TNF-α, plays a pivotal role during systemic inflammation. Excessive inflammation and TNF-α synthesis increase morbi-mortality in SS. In consequence, highly conserved endogenous mechanisms normally regulate the magnitude of innate immune response and prevent excessive inflammation (Wang *et al.*, 2003).

The CNS regulates systemic inflammatory responses to endotoxin through neural and humoral mechanisms. Evidence accumulated over the last 30 years suggests that norepinephrine (NE), the main neurotransmitter of the sympathetic nervous system, fulfills the criteria for neurotransmitter/neuromodulator in lymphoid organs: i) primary and secondary lymphoid organs receive extensive sympathetic/noradrenergic innervation; ii) under stimulation, NE is released from the sympathetic nerve terminals in these organs; and iii) the target immune cells, including lymphocytes and macrophages, express adrenergic receptors (AR). Adrenoceptors are G-protein coupled receptors that can be divided into two subgroups: the α- and β-AR, which can be further subdivided into different subtypes. Neutrophils, mononuclear, and natural killer cells, also T- and B-lymphocytes express α- and β-AR. The most important adrenoceptor –in terms of the immune system– is the β2-AR. Activation of β2-AR results in an increase in cAMP concentrations, which can modulate cytokine expression, *i.e.*, decreasing TNF-α and increasing IL-8 (Elenkov & Chrousos, 1999). However, recently was described that α2A-AR stimulation increases TNF-α gene expression in Kupffer cells and plasma TNF-α during sepsis (Miksa *et al.*, 2009). Thus, through AR stimulation, locally released NE, or circulating catecholamines, affect lymphocyte traffic, circulation, and proliferation, and modulate cytokine production and the functional activity of different lymphoid cells (Elenkov *et al.*, 2000), just as they control heart rate and other vital functions. Serum levels of sympatoadrenergic transmitters –*i.e.*, Neuropeptide-Y, ATP, and vanillyl mandelic acid (VMA, as an indirect measurement of catecholamine levels)–, are also increased during sepsis (Donoso *et al.*, 2008).

A growing body of literature is aimed at studying β-blockade as a treatment of sepsis. Their effects on metabolism and glucose homeostasis, cytokine expression, and myocardial function may be beneficial in the setting of sepsis. Sepsis induces an overall catabolic state, mainly due to excessive adrenergic stimulation (Bergmann *et al.*, 1999). β-Blockade has been proposed as a strategy to counteract the devastating consequences of this hyperadrenergic state. But treating a potentially hypotensive condition with a drug with antihypertensive properties may initially seem detrimental (Novotny *et al.*, 2009). Peripheral (i.p.) β1-AR blockade prior to endotoxemia increases survival time, reduces hepatic expression of pro-inflammatory cytokines, decreases protein expression of cardiac dysfunction markers, and preserves arterial blood pressure and left ventricular contractility (Ackland *et al.*, 2010). Surprisingly, few studies report overall mortality in the published β-blocker trials in sepsis. Interestingly, of those investigators that do report mortality in sepsis models, one out of four show increased mortality in β-blockade groups.

Vasopressor and inotropic therapies for sepsis employ adrenergic support. In fact, a recent publication about the "*Efficacy and Safety of Dopamine Versus Norepinephrine in the Management of Septic Shock*" showed that NE treatment decreases 28-day mortality and has a lower risk of sinus tachycardia and arrhythmias than dopamine (DA) (Patel *et al.*, 2010). This work concludes that arrhythmias are significant predictors of sepsis morbi-mortality, and that "*patients receiving DA should be monitored for the development of cardiac arrhythmias*", but does not consider a potential increase of MOD indicators induced by DA infusion, since high doses of DA (> 20 μg/kg/min) has a predominant α-AR effect, a potent

immunostimulator (Povoa & Carneiro, 2010). Recently De Baker *et al.* reported that DA administration is associated with greater mortality and a higher incidence of arrhythmic events compared to NE administration (De Backer *et al.*, 2012).

It should be noted that different cathecholamines used to treat patients with septic shock, have relative α- and β-AR effects (depending on the dose). Thus, in addition to individual differences, it is necessary to consider the fine-tuning of both, immune system and cardiovascular effects of adrenergic drugs used for sepsis treatment.

The CNS can also rapidly inhibit the release of macrophage TNF-α, and attenuate systemic inflammatory responses acting through the vagus (parasympathetic) nerve. This physiological mechanism, termed the 'cholinergic anti-inflammatory pathway (Borovikova *et al.*, 2000)' has major implications in immunology and in therapeutics (Rosas-Ballina & Tracey, 2009). The main vagal neurotransmitter, acetylcholine (ACh), inhibits LPS-induced TNF-α, IL-1β and IL-6 release, but not anti-inflammatory cytokine IL-10, in LPS stimulated *in vitro* cultured human macrophages (Borovikova *et al.*, 2000; Wang *et al.*, 2003). In addition, peripheral vagus nerve electrical stimulation inhibits liver TNF-α production, attenuates peak serum TNF-α amounts, and prevents the development of shock, during lethal endotoxemia in rats (Borovikova *et al.*, 2000).

Recent work on the anatomical basis of the cholinergic anti-inflammatory pathway indicates that the spleen is required for vagus nerve control of inflammation (Huston *et al.*, 2006). The spleen is the major source of serum TNF-α during endotoxemia (Mignini *et al.*, 2003). In splenectomized rats injected with endotoxin, serum TNF-α is reduced by 70%, and vagus nerve stimulation fails to further suppress TNF-α. The celiac branches of the vagus terminate in the celiac-superior mesenteric plexus and not in the spleen (Berthoud & Powley, 1996). The spleen is innervated by the splenic nerve, which originates in celiac-superior mesenteric plexus. The splenic nerve is composed mainly by catecholaminergic fibers, which terminate in close apposition to immune cells (Felten *et al.*, 1987). Thus, attenuation of TNF-α production by spleen macrophages induced by vagus nerve stimulation is mediated by norepinephrine released from splenic nerve endings. These data confirms the importance of the adrenergic transmitters in the regulation of immune response. It must be noted that immune cells have all the essential components of a non-neuronal cholinergic system and that ACh synthesized and released from lymphocytes acts as an immunomodulator via both muscarinic (mAChR) and nicotinic ACh receptors (nAChR) (Kawashima & Fujii, 2000; Kawashima & Fujii, 2003). Most evidences points towards a crucial role for the α7 nAChR in the cholinergic regulation of macrophage activity (Wang *et al.*, 2003). Nicotine exerts anti-inflammatory effects through α7 nAChR (Ulloa, 2005). Acetylcholine (and nicotine), also has cardiorespiratory effects (Fernandez *et al.*, 2002; Zapata *et al.*, 2002). Acting through the peripheral arterial chemoreceptors, ACh, nicotine, and epibatidine (a selective agonist for neuronal nAChRs) increases tidal volume and blood pressure in anesthetized cats (Zapata *et al.*, 2003; Reyes *et al.*, 2007), which support the idea that cholinergic nicotinic treatment can also improve cardiorespiratory performance during sepsis, and prevent tissue dysoxia, lactic acidosis and MODS. In addition, nicotine inhibit cardiac apoptosis induced by LPS in rats (Suzuki *et al.*, 2003).

Finally, both endotoxin and cytokines, stimulates HPA anti-inflammatory responses, either by adrenal glucocorticoids (Turnbull & Rivier, 1999) or by inhibiting prolactin secretion, a potent regulator of humoral and cellular immune response during physiological and pathological states (Freeman *et al.*, 2000). Thus, it is clear that the nervous system reflexively regulates the inflammatory response in real time, just as it controls heart rate and other vital functions.

6. Reflex control of inflammation: Part II – Immune-to-brain communication

Much less is known about the effect of the immune system on the CNS. Immune system-derived signals act on the CNS through four different pathways: i) by saturable transport across the blood–brain barrier (BBB) (Banks & Kastin, 1987); ii) by brain circumventricular organs (CVOs) (Stitt, 1990); iii) by cytokine binding to brain endothelial cells, which evokes paracrine mediators release (Fabry *et al.*, 1993; Cao *et al.*, 1998); and iv) by the activation of peripheral sensory nerves (i.e., vagus nerve) (Goehler *et al.*, 1997).

The role of peripheral sensory nerves in immunomodulation is controversial. It is believed that chemosensory transduction begins in immune cells, which release inflammatory mediators to activate neural elements, including vagal paraganglia (Goehler *et al.*, 1997; Goehler *et al.*, 1999) and primary afferent neurons located in sensory ganglia, which evokes host defense reflexes. Two cell types compose vagal paraganglia: type I (glomus) cells and type II (sustentacular) cells (Berthoud *et al.*, 1995). Vagal glomus cells (GC) are innervated by vagal afferent neurons, whose cell bodies are located in the nodose ganglion, and their central projection end primarily within the dorsal vagal complex (DVC) of the *medulla oblongata*. Thus, immunosensory inputs could initiate local cardiorespiratory reflexes and carry information about the state of inflammation.

In spite of the interleukin-1 (IL-1) receptor expression in vagal GC (Goehler *et al.*, 1997), IL-1β (and TNF-α), had no significant effect on the frequency of action potentials recorded from single fibers from isolated superfused rat GC obtained from vagal nerve paraganglia (Mac Grory *et al.*, 2010). In addition, in rodents exposed to i.p. LPS or IL-1β, bilateral subdiaphragmatic vagotomy prevents sickness manifestations and activation of *nucleus tractus solitarii* (NTS), *locus coeruleus* (LC), and hypothalamus (Bluthe *et al.*, 1994; Bret-Dibat *et al.*, 1995; Gaykema *et al.*, 1995; Watkins *et al.*, 1995; Hansen & Krueger, 1997; Borsody & Weiss, 2005). Thus, immune chemosensory inputs and incoming neural signals could be originated from other receptors, such as the peripheral arterial chemoreceptors neural pathway: the carotid body (CB) and its sensory ganglion.

The DVC consists of the NTS, the dorsal motor nucleus of the vagus (DMN), and the *area postrema* (AP) (Berthoud & Neuhuber, 2000). The DMN is the main site of origin of preganglionic vagus efferent fibers; while cardiovascular vagal efferences originate within the medullar *nucleus ambiguus* (NA). The AP, which lacks of BBB, is an important CVO and an important site for humoral immune-to-brain communication. The main portion of vagal

sensory input is received by neurons in the NTS, which coordinate autonomic function and interaction with the endocrine system. Ascending projections from the NTS reach hypothalamic paraventricular nucleus (PVN), an important structure in the HPA axis activation. Synaptic contacts also exist between the neurons in the NTS and rostral ventrolateral *medulla* (RVM), which occupies an important role in control of cardiovascular and respiratory homeostasis. The neurons from RVM project to the *locus coeruleus* (LC), which innervates higher brain sites, like hypothalamus and PVN. Neuronal projections emanate from the RVM and LC to sympathetic preganglionic neurons in the spinal cord. There are also descending pathways from the PVN to the RVM and NTS (Pavlov *et al.*, 2003). Thus, these ascending and descending connections provide a neuronal substrate for interaction between HPA axis and the ANS as an immunomodulatory mechanism.

In response to plasma levels of TNF-α, vagal immunosensory activity increases (Emch *et al.*, 2000) or decreases (Emch *et al.*, 2002) vagal motor activity. Transection of abdominal vagal trunks suppresses fever and hyperalgesia caused by i.p. LPS but has little effect on the febrile response to i.v. or intramuscular LPS. To elucidate the importance of visceral afferent innervation on the response to LPS, Wan *et al.* studied the expression of the immediate early gene c-fos in the hypothalamus and brain stem of the rat following peripheral –either i.v. or i.p.– injection of LPS. Subdiaphragmatic vagotomy completely blocked the induction of c-Fos protein following i.p. injection of LPS; however, vagotomy had a minimal effect on c-Fos protein induction following i.v. LPS administration (Wan *et al.*, 1994). In addition, c-Fos activation of NTS neurons induced by LPS persists after cervical bilateral vagotomy (Hermann *et al.*, 2001). Both subdiaphragmatic and cervical bilateral vagotomy abolition of CNS c-Fos activation induced by i.p. LPS are controversial, since it could be due to the section of neurons from the abdominal region that mediate the response to LPS *per se* or, merely, because of the role played by the vagus efferent fibers –perhaps those within the celiac branches– in LPS transport from the peritoneal cavity to the blood. Thus, when these fibers are cut, LPS escape to systemic circulation is limited, and systemic responses to LPS would be diminished (*e.g.*, c-Fos protein induction in the CNS) (Lenczowski *et al.*, 1997; Romanovsky *et al.*, 2000).

The number of neurons within the DVC that expressed c-Fos activation after peripheral administration of LPS is correlated with plasma levels of TNF-α. Thus, the activation of DVC neurons did not require intact vagal pathways, suggesting that TNF-α generated peripherally could acts directly on these neurons, because DVC exhibits the characteristics of CVOs (*i.e.*, fenestrated capillary network and absence of functional BBB) (Hermann *et al.*, 2001) or, more probably, through another neural afferent pathway. In consequence, it is possible to suggest that prominent CNS manifestations of endotoxemia are apparently caused by incoming neural signals provided by other peripheral receptors, distinct from vagal paraganglia, like the carotid arterial chemoreceptors, which function is intact after bilateral cervical vagotomy. Our results shown that LPS-induced c-Fos activation in NTS neurons and plasmatic cortisol increases in septic rats (treated i.p. with 15 mg/kg LPS) are suppressed by bilateral carotid neurotomy (Reyes *et al.*, 2012. Adv Exp Med Biol. *In press*) (Table 1).

Seen from an anatomical standpoint, the carotid body (CB) is the largest paraganglia in the body (Mascorro & Yates, 1980), and like other paraganglia, it receives sensory innervation, and has specialized glomus cells with abundant synapses with the sensory nervous fibers (Verna, 1997).

7. The arterial chemoreceptors in neuroimmunomodulation

The CB is the main peripheral chemoreceptor responsible for the detection of blood oxygen levels. The CB consists of groups of glomus (type I) cells arranged around capillaries, ensheathed by sustentacular (type II) cells, and surrounded by connective tissue. It receives profuse sensory innervation from the carotid (sinus) nerve (CSN), a branch of the glossopharyngeal nerve, whose sensory nerve endings are in close contact with glomus cells (GC) (Hess & Zapata, 1972). CB innervation is essentially by sensory neurons residing mainly in the petrosal ganglion (Kalia & Davies, 1978; Berger, 1980). Interestingly, the first synapse at the CNS for afferent CSN fibers occurs in the NTS (Donoghue *et al.*, 1984; Finley & Katz, 1992). Thus, inflammation-derived sensory input originated from arterial chemoreceptors (Zapata *et al.*, 2011) can be differentially processed in the peripheral chemoreceptor *per se*, in the sensory ganglion, and/or in the brainstem, and modify cardiorespiratory chemoreflexes, endocrine, and autonomic functions, like the neural control on the immune system. In rats, petrosal ganglion is a constituent of a ganglion complex, composed by nodose, petrosal and jugular ganglia, the nodose-petrosal-jugular ganglion complex (NPJgc).

Many reports allow us to propose that peripheral arterial chemoreceptors play a pivotal role in afferent signaling during sepsis. Recently, we demonstrated that i.v. administration of LPS to pentobarbitone-anesthetized cats evokes similar symptoms to those observed in patients with severe sepsis and septic shock, with tachycardia, tachypnea and hypotension, and that the increased respiratory frequency is prevented by bilateral section of the carotid and aortic nerves (Fernandez *et al.*, 2008). In addition, LPS enhances tonic CB chemosensory activity (measured by recording the frequency of chemosensory discharges) but reduces its responsiveness to transient excitatory (hypoxia and nicotine) or depressant (pure oxygen) stimuli. Diminished ventilatory responses to moderate and severe hypoxia in cats reproduces the diminished ventilatory responses to hypoxia observed in unanesthetized newborn piglets subjected to *Escherichia coli* endotoxin infusion (McDeigan *et al.*, 2003), as well as in rats, in a process that is in part mediated by an inhibitory effect of endothelial nitric oxide on the respiratory control mechanisms (Ladino *et al.*, 2007). Apoptosis studies carried out in CB excised from endotoxemic cats discard that CB diminished chemosensory activity observed in LPS-treated animals resulted from a reduction of functional tissue (Fernandez *et al.*, 2008), and suggest the participation of systemic soluble factors (*e.g.*, cytokines), or locally produced by either resident monocytes/macrophages (Dvorakova *et al.*, 2000), or parenchyma cells.

Lipopolysaccharide administration increases cytokine plasma levels in many species, including rats (Waage, 1987), bovines (Ohtsuka *et al.*, 1997) and cats (Otto & Rawlings, 1995). Thus, by using *in vitro* experiments, where the carotid artery is perfused and the entire preparation (including the CB) is superfused, the frequency of carotid nerve discharges

recorded under normoxic conditions was not significantly modified by TNF-α, but the enhanced CB chemosensory discharges recorded along responses to hypoxic stimulation was transiently diminished, in a dose-dependent manner (Fernandez *et al.*, 2008). The cat CB expresses both type-1 and type-2 TNF-α receptor mRNA. Immunohistochemical studies with specific antibodies, determined that TNF-R1 protein is located mainly in the GC. In addition, a strong positive TNF-α protein immunoreactivity was also found in the GC cytoplasm (Fernandez *et al.*, 2008). These observations suggest that locally or systemically produced and secreted TNF-α, acting in an autocrine or paracrine fashion, could modify GC function.

Apart from the presence of TNF-α and TNF-R1, it is known that GC from rat CB express IL-1 receptor type I (Wang *et al.*, 2002) and IL-6 receptor α (Wang *et al.*, 2006), and that GC respond to IL-1β application with depolarization and a transient rise in intracellular calcium (Shu *et al.*, 2007). On the other hand, i.p. administration of IL-1β evokes IL-1 receptor type I and tyrosine hydroxylase (TH) up-regulation in the rat CB (Zhang *et al.*, 2007). The fact that pro-inflammatory cytokines and their receptors are functionally expressed in the CB type I cells, suggests that inflammatory mediators may have different functional roles in the activation of neurons in the NPJgc, even in the absence of sepsis syndromes –*e.g.*, exerting a tonic control of cardiorespiratory, endocrine, autonomic, and/or immune functions–. In fact, hypoxia, the natural stimulus for peripheral arterial chemoreceptors upregulates the expression and function of proinflammatory cytokines in the rat CB (Lam *et al.*, 2008), and the adaptation to chronic hypoxia involves immune cell invasion and increased expression of inflammatory cytokines in rat CB (Liu *et al.*, 2009). Thus, it is possible to suggest that local or systemic pro-inflammatory cytokines, recognized by membrane receptors located in the GC, modify CB chemosensory activity and, through afferent pathways projecting to the NTS, stimulate or inhibit specific components of the systemic inflammatory response. It must be noted that, regarding the source of immune signals, neural pathways provide faster and more precise information than humoral pathways.

In view of data mentioned above, we tested whether LPS-induced systemic inflammation exerts a direct effect upon CB chemoreceptors. We determined that the rat CB and NPJgc constitutively express the mRNAs for TLR4, MyD88, TNF-α and its receptors (TNF-R1 and TNF-R2). Intraperitoneal administration of 15 mg/kg LPS evokes IKκB degradation, and subsequent NF-κB p65 translocation into the nucleus from GC and NPJgc chemosensory neurons. LPS also evokes p38 MAPK and ERK phosphorylation. Consistently, LPS treatment increases both mRNA and protein levels of TNF-α, TNF-R2, and TH. Double-labeling studies show that TLR4, TNF-α, and TNF-R1 are localized in TH-containing GC and neurons from CB and NPJgc, respectively, suggesting that the expression was confined to the chemoafferent neural pathway. TNF-R2 is also present surrounding GC clusters within the CB and in chemosensitive neurons. TNF-α, and TNF-R2 expression are increased in the carotid chemoreceptors from endotoxemic rats (Fernandez *et al.*, 2011). Thus –in addition to systemic LPS effect– our results suggest that LPS acting directly through TLR-4 modifies TNF-α and its receptors expression on chemosensory cells of the carotid chemoreceptors neural pathway. These results show a novel afferent pathway to the CNS during physiological conditions and endotoxemia, and could be relevant in understanding sepsis pathophysiology and therapy.

Thus, it is very interesting to highlight that during sepsis syndromes, LPS acting directly upon carotid chemoreceptors, modify TNF-α expression. In addition systemic or local inflammatory mediators could change arterial chemoreceptors function and afferent signaling through TNF-α receptors, whose expression is also modified during sepsis (our results), or through IL-1 and/or IL-6 receptors (Figure 1). Interestingly, TNF-α stimulates c-Fos activation of neurons in the NTS (Hermann *et al.*, 2001). Results here obtained would imply that arterial chemoreflexes, not only serves as a chemoreceptor for respiratory reflex responses, as traditionally accepted, but also as a sensor for the immune status, as modulator of autonomic balance tending to coordinate cardiorespiratory interplay devoted to maintain oxygen homeostasis in different pathologies, and as a protective factor during sepsis and MODS.

Figure 1. Proposed model for neural reflex control of inflammation during sepsis syndromes. Lypopolysaccharide (LPS) acting through macrophages or other cytokine-producing cell, increases plasma levels of pro-inflammatory cytokines (*e.g.,* TNF-α) which in activates immunosensory inputs from vagal paraganglia or carotid body (CB) chemoreceptors. Immunosensory signals reach the *nucleus tractus solitarii* (NTS) neurons. The dorsal motor nucleus (DMN) is the main site of origin of preganglionic vagus efferent fibers, activating the cholinergic anti-inflammatory reflex, by secreting acetylcholine (ACh), which decreased immune response. The main portion of vagal sensory inputs received by NTS neurons coordinates autonomic function and interaction with the endocrine system. Ascending projections from the NTS reach hypothalamic paraventricular nucleus (PVN), activating the hypothalamic-pituitary-adrenal (HPA) axis for glucocorticoids production and immunosupression. Synaptic contacts with the rostral

ventrolateral *medulla* (RVM) and subsequent projection to the *locus coeruleus* (LC) innervates higher brain sites, like PVN. Also, neuronal projections emanate from the RVM and LC to sympathetic preganglionic neurons in the spinal cord, which in turn activates adrenal epinephrine (EN) secretion and norepinephrine (NE), reducing pro-inflammatory activity. Thus, these ascending and descending connections provide a neuronal substrate for interaction between HPA axis and the ANS as an immunomodulatory mechanism. PG, petrosal ganglion; NG, nodose ganglion; CSN, carotid/sinus nerve; VN, vagus nerve; GPN, glossopharyngeal nerve; NS, nervous system; ACTH, adrenocorticotrophic hormone.

The disruption of continuous detection of the 'inflammatory status' of the body exerted by carotid chemoreceptors could be responsible for modifying the activity of the ANS, thus altering the control exerted by the nervous system on the immune system, and evoking an uncontrolled cytokine production. This excessive and uncontrolled systemic inflammatory response and dysautonomy could be responsible for subsequent neural uncoupling of the vital organs and MODS.

8. Conclusion

Sepsis syndromes are the main cause of death between critical care patients. They result from neural, cardiovascular, respiratory, and immune systems uncoupling. Multiple organ dysfunction syndrome (MODS) is due to an uncontrolled release of pro-inflammatory mediators, which damage parenchymatous organs. However, it is still unknown why sepsis progresses to MODS in only certain individuals.

The effects of sepsis therapies are controversial and strongly dependent of individual components, like individual response and genetic predisposition. Thus, the course of sepsis and therapies outcomes depends largely from host factors.

Increasing evidences shown that peripheral carotid chemoreceptors act as sensor for the immune status, as modulator of autonomic balance tending to coordinate cardiorespiratory interplay devoted to maintain oxygen homeostasis in different pathologies, and as a protective factor during sepsis and MODS.

As result of the autonomic and immune imbalance originated from carotid chemoreceptors, neural and cytokine communication networks between healthy organs are disrupted. So, the impaired autonomic function would decrease cardiorespiratory function, oxygen delivery to the tissues, and the reflex control of inflammation. The heterostasis induced by systemic inflammation worsens the uncoupling of biological oscillators, what would lead to MODS and death.

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Acknowledgement

9. References

Abboud FM & Thames MD. Interaction of cardiovascular reflexes in circulatory control. Sheperd, J. T, Abboud, F. M, Geiger, S. R, and Bethesda, M. D. [3], 675-752. 1983. American Physiological Society. Handbook of Physiology.

Ackland GL, Yao ST, Rudiger A, Dyson A, Stidwill R, Poputnikov D, Singer M, & Gourine AV (2010). Cardioprotection, attenuated systemic inflammation, and survival benefit of beta1-adrenoceptor blockade in severe sepsis in rats. *Crit Care Med* 38, 388-394.

Akashi-Takamura S & Miyake K (2008). TLR accessory molecules. *Curr Opin Immunol* 20, 420-425.

Akira S, Takeda K, & Kaisho T (2001). Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2, 675-680.

Akira S, Uematsu S, & Takeuchi O (2006). Pathogen recognition and innate immunity. *Cell* 124, 783-801.

Alanis J, Defillo B, & Gordon S (1968). Changes in the efferent discharges of sympathetic and parasympathetic cardiac nerves provoked by activation of carotid chemoreceptors. *Arch Int Physiol Biochim* 76, 214-235.

Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, & Pinsky MR (2001). Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 29, 1303-1310.

Banks WA & Kastin AJ (1987). Saturable transport of peptides across the blood-brain barrier. *Life Sci* 41, 1319-1338.

Barnaby D, Ferrick K, Kaplan DT, Shah S, Bijur P, & Gallagher EJ (2002). Heart rate variability in emergency department patients with sepsis. *Acad Emerg Med* 9, 661-670.

Barochia AV, Cui X, Vitberg D, Suffredini AF, O'Grady NP, Banks SM, Minneci P, Kern SJ, Danner RL, Natanson C, & Eichacker PQ (2010). Bundled care for septic shock: an analysis of clinical trials. *Crit Care Med* 38, 668-678.

Berger AJ (1980). The distribution of the cat's carotid sinus nerve afferent and efferent cell bodies using the horseradish peroxidase technique. *Brain Res* 190, 309-320.

Bergmann M, Gornikiewicz A, Sautner T, Waldmann E, Weber T, Mittlbock M, Roth E, & Fugger R (1999). Attenuation of catecholamine-induced immunosuppression in whole blood from patients with sepsis. *Shock* 12, 421-427.

Berthoud HR, Kressel M, & Neuhuber WL (1995). Vagal afferent innervation of rat abdominal paraganglia as revealed by anterograde DiI-tracing and confocal microscopy. *Acta Anat (Basel)* 152, 127-132.

Berthoud HR & Neuhuber WL (2000). Functional and chemical anatomy of the afferent vagal system. *Auton Neurosci* 85, 1-17.

Berthoud HR & Powley TL (1996). Interaction between parasympathetic and sympathetic nerves in prevertebral ganglia: morphological evidence for vagal efferent innervation of ganglion cells in the rat. *Microsc Res Tech* 35, 80-86.

Bluthe RM, Walter V, Parnet P, Laye S, Lestage J, Verrier D, Poole S, Stenning BE, Kelley KW, & Dantzer R (1994). Lipopolysaccharide induces sickness behaviour in rats by a vagal mediated mechanism. *C R Acad Sci III* 317, 499-503.

Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, & Sibbald WJ (1992). Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 101, 1644-1655.

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

Borsody MK & Weiss JM (2005). The subdiaphragmatic vagus nerves mediate activation of locus coeruleus neurons by peripherally administered microbial substances. *Neuroscience* 131, 235-245.

Bret-Dibat JL, Bluthe RM, Kent S, Kelley KW, & Dantzer R (1995). Lipopolysaccharide and interleukin-1 depress food-motivated behavior in mice by a vagal-mediated mechanism. *Brain Behav Immun* 9, 242-246.

Cao C, Matsumura K, Yamagata K, & Watanabe Y (1998). Cyclooxygenase-2 is induced in brain blood vessels during fever evoked by peripheral or central administration of tumor necrosis factor. *Brain Res Mol Brain Res* 56, 45-56.

Carre JE & Singer M (2008). Cellular energetic metabolism in sepsis: the need for a systems approach. *Biochim Biophys Acta* 1777, 763-771.

Chalupka AN & Talmor D (2012). The economics of sepsis. *Crit Care Clin* 28, 57-76, vi.

Chen WL, Chen JH, Huang CC, Kuo CD, Huang CI, & Lee LS (2008). Heart rate variability measures as predictors of in-hospital mortality in ED patients with sepsis. *Am J Emerg Med* 26, 395-401.

Crouser ED (2004). Mitochondrial dysfunction in septic shock and multiple organ dysfunction syndrome. *Mitochondrion* 4, 729-741.

da Silva CJ, Soldau K, Christen U, Tobias PS, & Ulevitch RJ (2001). Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex. transfer from CD14 to TLR4 and MD-2. *J Biol Chem* 276, 21129-21135.

De Backer D, Aldecoa C, Njimi H, & Vincent JL (2012). Dopamine versus norepinephrine in the treatment of septic shock: a meta-analysis*. *Crit Care Med* 40, 725-730.

Dellinger RP, Carlet JM, Masur H, Gerlach H, Calandra T, Cohen J, Gea-Banacloche J, Keh D, Marshall JC, Parker MM, Ramsay G, Zimmerman JL, Vincent JL, & Levy MM (2004).

Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. *Crit Care Med* 32, 858-873.

Donoghue S, Felder RB, Jordan D, & Spyer KM (1984). The central projections of carotid baroreceptors and chemoreceptors in the cat: a neurophysiological study. *J Physiol* 347, 397-409.

Donoso V, Gomez CR, Orriantia MA, Perez V, Torres C, Coddou C, Nelson P, Maisey K, Morales B, Fernandez R, Imarai M, Huidobro-Toro JP, Sierra F, & Acuna-Castillo C (2008). The release of sympathetic neurotransmitters is impaired in aged rats after an inflammatory stimulus: a possible link between cytokine production and sympathetic transmission. *Mech Ageing Dev* 129, 728-734.

Dvorakova M, Hohler B, Vollerthun R, Fischbach T, & Kummer W (2000). Macrophages: a major source of cytochrome b558 in the rat carotid body. *Brain Res* 852, 349-354.

Elenkov IJ & Chrousos GP (1999). Stress Hormones, Th1/Th2 patterns, Pro/Anti-inflammatory Cytokines and Susceptibility to Disease. *Trends Endocrinol Metab* 10, 359-368.

Elenkov IJ, Wilder RL, Chrousos GP, & Vizi ES (2000). The sympathetic nerve--an integrative interface between two supersystems: the brain and the immune system. *Pharmacol Rev* 52, 595-638.

Emch GS, Hermann GE, & Rogers RC (2002). Tumor necrosis factor-alpha inhibits physiologically identified dorsal motor nucleus neurons in vivo. *Brain Res* 951, 311-315.

Emch GS, Hermann GE, & Rogers RC (2000). TNF-alpha activates solitary nucleus neurons responsive to gastric distension. *Am J Physiol Gastrointest Liver Physiol* 279, G582-G586.

Eyzaguirre C, Fitzgerald RS, & Lahiri S. Arterial Chemoreceptors. Sheperd, J. T, Abboud, F. M, Geiger, S. R, and Bethesda, M. D. [3], 557-662. 1983. American Physiological Society. Handbook of Physiology. Ref Type: Serial (Book,Monograph)

Fabry Z, Fitzsimmons KM, Herlein JA, Moninger TO, Dobbs MB, & Hart MN (1993). Production of the cytokines interleukin 1 and 6 by murine brain microvessel endothelium and smooth muscle pericytes. *J Neuroimmunol* 47, 23-34.

Felten DL, Ackerman KD, Wiegand SJ, & Felten SY (1987). Noradrenergic sympathetic innervation of the spleen: I. Nerve fibers associate with lymphocytes and macrophages in specific compartments of the splenic white pulp. *J Neurosci Res* 18, 28-21.

Fernandez R, Gonzalez S, Rey S, Cortes PP, Maisey KR, Reyes EP, Larrain C, & Zapata P (2008). Lipopolysaccharide-induced carotid body inflammation in cats: functional manifestations, histopathology and involvement of tumour necrosis factor-alpha. *Exp Physiol* 93, 892-907.

Fernandez R, Larrain C, & Zapata P (2002). Acute ventilatory and circulatory reactions evoked by nicotine: are they excitatory or depressant? *Respir Physiol Neurobiol* 133, 173-182.

Fernandez R, Nardocci G, Simon F, Martin A, Becerra A, Rodriguez-Tirado C, Maisey KR, cuna-Castillo C, & Cortes PP (2011). Lipopolysaccharide signaling in the carotid chemoreceptor pathway of rats with sepsis syndrome. *Respir Physiol Neurobiol* 175, 336-348.

Finley JC & Katz DM (1992). The central organization of carotid body afferent projections to the brainstem of the rat. *Brain Res* 572, 108-116.

Freeman ME, Kanyicska B, Lerant A, & Nagy G (2000). Prolactin: structure, function, and regulation of secretion. *Physiol Rev* 80, 1523-1631.

Gaykema RP, Dijkstra I, & Tilders FJ (1995). Subdiaphragmatic vagotomy suppresses endotoxin-induced activation of hypothalamic corticotropin-releasing hormone neurons and ACTH secretion. *Endocrinology* 136, 4717-4720.

Godin PJ & Buchman TG (1996). Uncoupling of biological oscillators: a complementary hypothesis concerning the pathogenesis of multiple organ dysfunction syndrome. *Crit Care Med* 24, 1107-1116.

Godin PJ, Fleisher LA, Eidsath A, Vandivier RW, Preas HL, Banks SM, Buchman TG, & Suffredini AF (1996). Experimental human endotoxemia increases cardiac regularity: results from a prospective, randomized, crossover trial. *Crit Care Med* 24, 1117-1124.

Goehler LE, Gaykema RP, Nguyen KT, Lee JE, Tilders FJ, Maier SF, & Watkins LR (1999). Interleukin-1beta in immune cells of the abdominal vagus nerve: a link between the immune and nervous systems? *J Neurosci* 19, 2799-2806.

Goehler LE, Relton JK, Dripps D, Kiechle R, Tartaglia N, Maier SF, & Watkins LR (1997). Vagal paraganglia bind biotinylated interleukin-1 receptor antagonist: a possible mechanism for immune-to-brain communication. *Brain Res Bull* 43, 357-364.

Hansen MK & Krueger JM (1997). Subdiaphragmatic vagotomy blocks the sleep- and fever-promoting effects of interleukin-1beta. *Am J Physiol* 273, R1246-R1253.

Hermann GE, Emch GS, Tovar CA, & Rogers RC (2001). c-Fos generation in the dorsal vagal complex after systemic endotoxin is not dependent on the vagus nerve. *Am J Physiol Regul Integr Comp Physiol* 280, R289-R299.

Hess A & Zapata P (1972). Innervation of the cat carotid body: normal and experimental studies. *Fed Proc* 31, 1365-1382.

Hotchkiss RS & Karl IE (2003). The pathophysiology and treatment of sepsis. *N Engl J Med* 348, 138-150.

Howell MD, Donnino M, Clardy P, Talmor D, & Shapiro NI (2007). Occult hypoperfusion and mortality in patients with suspected infection. *Intensive Care Med* 33, 1892-1899.

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 (2006). Splenectomy inactivates the cholinergic antiinflammatory pathway during lethal endotoxemia and polymicrobial sepsis. *J Exp Med* 203, 1623-1628.

Kalia M & Davies RO (1978). A neuroanatomical search for glossopharyngeal efferents to the carotid body using the retrograde transport of horseradish peroxidase. *Brain Res* 149, 477-481.

Kawashima K & Fujii T (2000). Extraneuronal cholinergic system in lymphocytes. *Pharmacol Ther* 86, 29-48.

Kawashima K & Fujii T (2003). The lymphocytic cholinergic system and its biological function. *Life Sci* 72, 2101-2109.

Kirchheim HR (1976). Systemic arterial baroreceptor reflexes. *Physiol Rev* 56, 100-177.

Korach M, Sharshar T, Jarrin I, Fouillot JP, Raphael JC, Gajdos P, & Annane D (2001). Cardiac variability in critically ill adults: influence of sepsis. *Crit Care Med* 29, 1380-1385.

Ladino J, Bancalari E, & Suguihara C (2007). Ventilatory response to hypoxia during endotoxemia in young rats: role of nitric oxide. *Pediatr Res* 62, 134-138.

Lam SY, Tipoe GL, Liong EC, & Fung ML (2008). Chronic hypoxia upregulates the expression and function of proinflammatory cytokines in the rat carotid body. *Histochem Cell Biol* 130, 549-559.

Lenczowski MJ, Van Dam AM, Poole S, Larrick JW, & Tilders FJ (1997). Role of circulating endotoxin and interleukin-6 in the ACTH and corticosterone response to intraperitoneal LPS. *Am J Physiol* 273, R1870-R1877.

Libert C (2003). Inflammation: A nervous connection. *Nature* 421, 328-329.

Liljestrand A (1958). Neural control of respiration. *Physiol Rev* 38, 691-708.

Liu X, He L, Stensaas L, Dinger B, & Fidone S (2009). Adaptation to chronic hypoxia involves immune cell invasion and increased expression of inflammatory cytokines in rat carotid body. *Am J Physiol Lung Cell Mol Physiol* 296, L158-L166.

Mac Grory B, O'Connor ET, O'Halloran KD, & Jones JF (2010). The effect of pro-inflammatory cytokines on the discharge rate of vagal nerve paraganglia in the rat. *Respir Physiol Neurobiol* 171, 122-127.

Martin GS, Mannino DM, Eaton S, & Moss M (2003). The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 348, 1546-1554.

Martin GS, Mannino DM, & Moss M (2006). The effect of age on the development and outcome of adult sepsis. *Crit Care Med* 34, 15-21.

Martinez JD, Babu RV, & Sharma G (2009). Escherichia coli septic shock masquerading as ST-segment elevation myocardial infarction. *Postgrad Med* 121, 102-105.

Mascorro JA & Yates RD (1980). Paraneurons and paraganglia: histological and ultrastructural comparisons between intraganglionic paraneurons and extra-adrenal paraganglion cells. *Adv Biochem Psychopharmacol* 25, 201-213.

McDeigan GE, Ladino J, Hehre D, Devia C, Bancalari E, & Suguihara C (2003). The effect of Escherichia coli endotoxin infusion on the ventilatory response to hypoxia in unanesthetized newborn piglets. *Pediatr Res* 53, 950-955.

Medvedev AE, Kopydlowski KM, & Vogel SN (2000). Inhibition of lipopolysaccharide-induced signal transduction in endotoxin-tolerized mouse macrophages: dysregulation of cytokine, chemokine, and toll-like receptor 2 and 4 gene expression. *J Immunol* 164, 5564-5574.

Meregalli A, Oliveira RP, & Friedman G (2004). Occult hypoperfusion is associated with increased mortality in hemodynamically stable, high-risk, surgical patients. *Crit Care* 8, R60-R65.

Mignini F, Streccioni V, & Amenta F (2003). Autonomic innervation of immune organs and neuroimmune modulation. *Auton Autacoid Pharmacol* 23, 1-25.

Miksa M, Das P, Zhou M, Wu R, Dong W, Ji Y, Goyert SM, Ravikumar TS, & Wang P (2009). Pivotal role of the alpha(2A)-adrenoceptor in producing inflammation and organ injury in a rat model of sepsis. *PLoS One* 4, e5504.

Misset D, Martin C, Cariou A, Carlet J, Brun-Buisson C, & Annane D. Activated Protein C and Corticosteroids for Human Septic Shock (APROCCHS). 2010. 2010. Ref Type: Internet Communication

Montarolo PG, Passatore M, & Raschi F (1976). Carotid chemoreceptor influence on the cardiac sympathetic nerve discharge. *Experientia* 32, 480-481.

Natanson C, Danner RL, Reilly JM, Doerfler ML, Hoffman WD, Akin GL, Hosseini JM, Banks SM, Elin RJ, MacVittie TJ, & . (1990). Antibiotics versus cardiovascular support in a canine model of human septic shock. *Am J Physiol* 259, H1440-H1447.

Nguyen HB, Corbett SW, Steele R, Banta J, Clark RT, Hayes SR, Edwards J, Cho TW, & Wittlake WA (2007). Implementation of a bundle of quality indicators for the early management of severe sepsis and septic shock is associated with decreased mortality. *Crit Care Med* 35, 1105-1112.

Nguyen HB, Rivers EP, Knoblich BP, Jacobsen G, Muzzin A, Ressler JA, & Tomlanovich MC (2004). Early lactate clearance is associated with improved outcome in severe sepsis and septic shock. *Crit Care Med* 32, 1637-1642.

Novotny NM, Lahm T, Markel TA, Crisostomo PR, Wang M, Wang Y, Ray R, Tan J, Al-Azzawi D, & Meldrum DR (2009). beta-Blockers in sepsis: reexamining the evidence. *Shock* 31, 113-119.

Ohtsuka H, Ohki K, Tanaka T, Tajima M, Yoshino T, & Takahashi K (1997). Circulating tumor necrosis factor and interleukin-1 after administration of LPS in adult cows. *J Vet Med Sci* 59, 927-929.

Otto CM & Rawlings CA (1995). Tumor necrosis factor production in cats in response to lipopolysaccharide: an in vivo and in vitro study. *Vet Immunol Immunopathol* 49, 183-188.

Patel GP, Grahe JS, Sperry M, Singla S, Elpern E, Lateef O, & Balk RA (2010). Efficacy and safety of dopamine versus norepinephrine in the management of septic shock. *Shock* 33, 375-380.

Patel GP, Gurka DP, & Balk RA (2003). New treatment strategies for severe sepsis and septic shock. *Curr Opin Crit Care* 9, 390-396.

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

Perel A (2008). Bench-to-bedside review: the initial hemodynamic resuscitation of the septic patient according to Surviving Sepsis Campaign guidelines--does one size fit all? *Crit Care* 12, 223.

Peres BD, Melot C, Lopes FF, Nguyen B, V, & Vincent JL (2002). The Multiple Organ Dysfunction Score (MODS) versus the Sequential Organ Failure Assessment (SOFA) score in outcome prediction. *Intensive Care Med* 28, 1619-1624.

Pinsky MR (2004). Dysregulation of the immune response in severe sepsis. *Am J Med Sci* 328, 220-229.

Povoa P & Carneiro AH (2010). Adrenergic support in septic shock: a critical review. *Hosp Pract (Minneap)* 38, 62-73.

Rassias AJ, Holzberger PT, Givan AL, Fahrner SL, & Yeager MP (2005). Decreased physiologic variability as a generalized response to human endotoxemia. *Crit Care Med* 33, 512-519.

Reyes EP, Abarzua S, Martin A, Rodriguez J, Cortes PP & Fernandez R (2012) LPS-induced c-Fos activation in NTS neurons and plasmatic cortisol increases in septic rats are suppressed by bilateral carotid chemodenervation. *Adv Exp Med Biol*. In Press.

Reyes EP, Fernandez R, Larrain C, & Zapata P (2007). Carotid body chemosensory activity and ventilatory chemoreflexes in cats persist after combined cholinergic-purinergic block. *Respir Physiol Neurobiol* 156, 23-32.

Riedemann NC, Guo RF, & Ward PA (2003). The enigma of sepsis. *J Clin Invest* 112, 460-467.

Rogausch H, Vo NT, Del RA, & Besedovsky HO (2000). Increased sensitivity of the baroreceptor reflex after bacterial endotoxin. *Ann N Y Acad Sci* 917, 165-168.

Romanovsky AA, Ivanov AI, Berthoud HR, & Kulchitsky VA (2000). Are vagal efferents involved in the fever response to intraperitoneal lipopolysaccharide? *J Therm Biol* 25, 65-70.

Rosas-Ballina M & Tracey KJ (2009). Cholinergic control of inflammation. *J Intern Med* 265, 663-679.

Sanlioglu S, Williams CM, Samavati L, Butler NS, Wang G, McCray PB, Jr., Ritchie TC, Hunninghake GW, Zandi E, & Engelhardt JF (2001). Lipopolysaccharide induces Rac1-dependent reactive oxygen species formation and coordinates tumor necrosis factor-alpha secretion through IKK regulation of NF-kappa B. *J Biol Chem* 276, 30188-30198.

Schletter J, Heine H, Ulmer AJ, & Rietschel ET (1995). Molecular mechanisms of endotoxin activity. *Arch Microbiol* 164, 383-389.

Schmidt H, Muller-Werdan U, Hoffmann T, Francis DP, Piepoli MF, Rauchhaus M, Prondzinsky R, Loppnow H, Buerke M, Hoyer D, & Werdan K (2005). Autonomic dysfunction predicts mortality in patients with multiple organ dysfunction syndrome of different age groups. *Crit Care Med* 33, 1994-2002.

Schmidt H, Muller-Werdan U, Nuding S, Hoffmann T, Francis DP, Hoyer D, Rauchhaus M, & Werdan K (2004). Impaired chemoreflex sensitivity in adult patients with multiple organ dysfunction syndrome--the potential role of disease severity. *Intensive Care Med* 30, 665-672.

Schmidt HB, Heinroth K, & Werdan K (1999). Autonomic dysfunction in critically ill patients. In *Yearbokk of Intensive Care and En.ergency Medicine*, ed. Vincent JL, pp. 519-536. Springer, New York.

Schmidt HB, Werdan K, & Muller-Werdan U (2001). Autonomic dysfunction in the ICU patient. *Curr Opin Crit Care* 7, 314-322.

Schueller PO, Steiner S, Hennersdorf MG, & Strauer BE (2008). Cardiac chemoreflex sensitivity in critically ill patients. *J Physiol Pharmacol* 59 Suppl 6, 623-627.

Shen FM, Guan YF, Xie HH, & Su DF (2004). Arterial baroreflex function determines the survival time in lipopolysaccharide-induced shock in rats. *Shock* 21, 556-560.

Shu HF, Wang BR, Wang SR, Yao W, Huang HP, Zhou Z, Wang X, Fan J, Wang T, & Ju G (2007). IL-1beta inhibits IK and increases [Ca2+]i in the carotid body glomus cells and increases carotid sinus nerve firings in the rat. *Eur J Neurosci* 25, 3638-3647.

Simon F & Fernandez R (2009). Early lipopolysaccharide-induced reactive oxygen species production evokes necrotic cell death in human umbilical vein endothelial cells. *J Hypertens* 27, 1202-1216.

Singer M, De S, V, Vitale D, & Jeffcoate W (2004). Multiorgan failure is an adaptive, endocrine-mediated, metabolic response to overwhelming systemic inflammation. *Lancet* 364, 545-548.

Singh S & Evans TW (2006). Organ dysfunction during sepsis. *Intensive Care Med* 32, 349-360.

Somers VK, Mark AL, & Abboud FM (1991). Interaction of baroreceptor and chemoreceptor reflex control of sympathetic nerve activity in normal humans. *J Clin Invest* 87, 1953-1957.

Stitt JT (1990). Passage of immunomodulators across the blood-brain barrier. *Yale J Biol Med* 63, 121-131.

Suzuki J, Bayna E, Dalle ME, & Lew WY (2003). Nicotine inhibits cardiac apoptosis induced by lipopolysaccharide in rats. *J Am Coll Cardiol* 41, 482-488.

Tang GJ, Kou YR, & Lin YS (1998). Peripheral neural modulation of endotoxin-induced hyperventilation. *Crit Care Med* 26, 1558-1563.

Thaver D & Zaidi AK (2009). Burden of neonatal infections in developing countries: a review of evidence from community-based studies. *Pediatr Infect Dis J* 28, S3-S9.

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

Turnbull AV & Rivier CL (1999). Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiol Rev* 79, 1-71.

Ulloa L (2005). The vagus nerve and the nicotinic anti-inflammatory pathway. *Nat Rev Drug Discov* 4, 673-684.

van Dillen J, Zwart J, chutte J, & an RJ (2010). Maternal sepsis: epidemiology, etiology and outcome. *Curr Opin Infect Dis* 23, 249-254.

Vayssettes-Courchay C, Bouysset F, & Verbeuren TJ (2005). Sympathetic activation and tachycardia in lipopolysaccharide treated rats are temporally correlated and unrelated to the baroreflex. *Auton Neurosci* 120, 35-45.

ver Elst KM, Spapen HD, Nguyen DN, Garbar C, Huyghens LP, & Gorus FK (2000). Cardiac troponins I and T are biological markers of left ventricular dysfunction in septic shock. *Clin Chem* 46, 650-657.

Verna A (1997). The Mammalian Carotid Body: morphological data. In *The Carotid Body Chemoreceptors*, ed. González C, pp. 1-29. Springer, New York.

Volk T & Kox WJ (2000). Endothelium function in sepsis. *Inflamm Res* 49, 185-198.

Waage A (1987). Production and clearance of tumor necrosis factor in rats exposed to endotoxin and dexamethasone. *Clin Immunol Immunopathol* 45, 348-355.

Wan W, Wetmore L, Sorensen CM, Greenberg AH, & Nance DM (1994). Neural and biochemical mediators of endotoxin and stress-induced c-fos expression in the rat brain. *Brain Res Bull* 34, 7-14.

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 (2003). Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* 421, 384-388.

Wang X, Wang BR, Duan XL, Zhang P, Ding YQ, Jia Y, Jiao XY, & Ju G (2002). Strong expression of interleukin-1 receptor type I in the rat carotid body. *J Histochem Cytochem* 50, 1677-1684.

Wang X, Zhang XJ, Xu Z, Li X, Li GL, Ju G, & Wang BR (2006). Morphological evidence for existence of IL-6 receptor alpha in the glomus cells of rat carotid body. *Anat Rec A Discov Mol Cell Evol Biol* 288, 292-296.

Watkins LR, Goehler LE, Relton JK, Tartaglia N, Silbert L, Martin D, & Maier SF (1995). Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. *Neurosci Lett* 183, 27-31.

Watson RS & Carcillo JA (2005). Scope and epidemiology of pediatric sepsis. *Pediatr Crit Care Med* 6, S3-S5.

Yang FL, Li CH, Hsu BG, Tsai NM, Lin SZ, Harn HJ, Chen HI, Liao KW, & Lee RP (2007). The reduction of tumor necrosis factor-alpha release and tissue damage by pentobarbital in the experimental endotoxemia model. *Shock* 28, 309-316.

Yucel T, Memis D, Karamanlioglu B, Sut N, & Yuksel M (2008). The prognostic value of atrial and brain natriuretic peptides, troponin I and C-reactive protein in patients with sepsis. *Exp Clin Cardiol* 13, 183-188.

Zapata P, Larrain C, & Fernandez R (2002). Nicotine-evoked ventilatory reflexes in cats: sites of origin and afferents. *J Anat* 200, 204.

Zapata P, Larrain C, Fernandez R, & Reyes EP (2003). Cholinergic actions on carotid chemosensory system. *Adv Exp Med Biol* 536, 277-283.

Zapata P, Larrain C, Reyes P, & Fernandez R (2011). Immunosensory signalling by carotid body chemoreceptors. *Respir Physiol Neurobiol* 178, 370-374.

Zhang XJ, Wang X, Xiong LZ, Fan J, Duan XL, & Wang BR (2007). Up-regulation of IL-1 receptor type I and tyrosine hydroxylase in the rat carotid body following intraperitoneal injection of IL-1beta. *Histochem Cell Biol* 128, 533-540.