Journal of Internal Medicine 2005; **257**: 122–125

MINISYMPOSIUM

The inflammatory reflex – Introduction

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Abstract. Andersson J (Karolinska University Hospital Huddinge, Stockholm, Sweden). The inflammatory reflex – Introduction (Minisymposium). *J Intern Med* 2005; **257:** 122–125.

Sepsis is the third leading cause of death in the developed world. Despite recent advances in intensive care treatment and the discovery of antibiotics, sepsis remains associated with a high mortality rate. The pathogenesis of sepsis is characterized by an overwhelming systemic inflammatory response that is central to the development of lethal multiple organ failure. This volume of the *Journal of Internal Medicine* contains three reviews addressing novel aspects of a system we are only beginning to understand – the interactions between the immune and the nervous systems, the 'neuro-immune axis'. Tracey (*Nature* 2002; **420**: 853) recently discovered that the nervous system, through the vagus nerve, can modulate circulating TNF-α levels induced by microbial invasion or tissue injury. This cholinergic anti-inflammatory pathway is mediated primarily by nicotinic acetylcholine receptors on tissue macrophages – the pathway leads to decreased production of proinflammatory cytokines. The author reports that treatment with the acetylcholine receptor agonist, nicotine, modulates this system and reduces mortality in 'established' sepsis. Watkins and Maier (*J Intern Med* 2005; **257**: 139) illustrate that pathological pain (induced by inflammation) is not simply a strict neuronal phenomenon, but is a component of the immune response, and is modulated by peripheral immune cells and spinal cord glia cells. This may be of importance for future development of novel drugs for neuropathic pain as well as our understanding of increased risks for infections in anaesthetic skin areas. Blalock (*J Immunol* 1984; **132**: 1067) elucidates the possibility that the immune system actually functions as the sixth sense, sensing microbes and microbial toxins that we cannot see, hear, taste, touch or smell. Activation of the sympathetic nervous system also has predominantly anti-inflammatory effects that are mediated through direct nerve to immune cell interaction or through the adrenal neuro-endocrine axis.

Keywords: inflammation, cytokine, sepsis, neuro-immune modulation, pain.

Introduction

The innate immune system is activated by infection and injury to release pro-inflammatory cytokines, which stimulate macrophages and neutrophils and modulate specific cellular responses. The magnitude of the cytokine response is critical, because a deficient response may result in secondary infections, whilst an excessive response may be more injurious than the original insult. During the past 30 years we have gradually come to understand that cytokines act as both paracrine and endocrine mediators of inflammation: they co-ordinate local and systemic inflammatory responses. It is widely known that these responses are regulated via anti-inflammatory responses including glucocorticoids and counter-regulatory cytokines [e.g. interleukin (IL)-10]. The activity of these anti-inflammatory mediators can

protect the host from excessive damage. As with other cytokines, these 'classical' anti-inflammatory mediators act via a humoral system that is dependent upon the local concentration of the cytokine. Surprising new research has revealed that inflammation is also tightly controlled by nerves, particularly a newly identified autonomic neuronal pathway that reflexively monitors and adjusts the inflammatory response by inhibiting pro-inflammatory cytokine synthesis [1]. This inflammatory reflex pathway is rapid, localized and integrated in contrast to the well defined humoral regulation of inflammation. These findings represent the beginnings of a new dogma: the neuro-immune axis can controls inflammation via neural regulation of the immune response.

The immune system – the body's sixth sense?

The human body is colonized by at least 100-fold higher numbers of microbial cells when compared with our own eukaryotic cells. Clearly the mammalian immune system has become highly efficient in protection against mucosal and skin invasion of these potential pathogens. But what if the immune system is not acting alone in this defensive posture? Is it possible that nervous system monitors the front line activities of the immune system, like it monitors heart rate and other visceral functions? In this issue Blalock *et al.* consider this point, and elucidate the possibility that the immune system actually functions as the sixth sense, sensing microbes and microbial toxins that we cannot see, hear, taste, touch or smell [2]. Indeed recent studies delineate that the immune system communicates with the nervous system in a bi-directional way [3]. The activation of pituitary-dependent adrenal responses after endotoxin administration provided early evidence that inflammatory stimuli can activate anti-inflammatory signals from the central nervous system (CNS). Subsequently, Besedovsky *et al.* showed directly that inflammation in peripheral tissues alters neuronal signalling in the hypothalamus. Extensive work has identified a common molecular basis for communication, with cells from each system expressing signalling ligands and receptors from the other. For example, neurones in the CNS can synthesize and express tumour necrosis factor (TNF) and IL-1, and these cytokines may participate in neuronal communication. It is now evident that neuropeptides and cytokines are synthesized by immune cells as well as by the nervous tissues and serve as the molecular basis of a neuro-immune axis. Furthermore, the immune system can produce peptide hormones and neurotransmitters including acetylcholine, melatonin, adrenocorticotropin (ACTH) and endorphins whilst neuronal cells can produce IL-1. Thus, cooperation between the two systems [4], e.g. via these shared signalling molecules cause physiological responses that are ultimately beneficial to the host and detrimental to the invading infectious agents.

Blalock *et al.* have revealed that IFN-α not only provides antiviral activity but also generates analgesic effects via induction of endorphins. Single mutation studies demonstrate that these two biological functions of IFN-α are induced by specific different parts of the molecule [5]. As an example of the redundancy of the neuro-immune axis, they have also demonstrated that T lymphocytes can produce adrenocorticotropic hormone, thyroid-stimulating hormone, vasoactive intestinal peptide as well as insulin-like growth factor. Furthermore, macrophages can produce endorphins, growth hormone, substance P as well as ACTH and splenocytes can produce luteinizing hormone, follicle-stimulating hormone, corticotropin-releasing hormone as well as adrenaline and endorphins. This bi-directional crosstalk between the CNS and the immune system may explain how mild, perhaps imperceptible stress causes the hypothalamus to release cortico-releasing hormone in quantities too low to evoke pituitary ACTH release, but sufficient to upregulate pituitary IL-1 receptors. If this coincides with an infection or inflammation that elicit more IL-1, the pituitary may be triggered to excessive ACTH release and a stress response that is above and beyond what would normally be expected for that level of initial microbial insult. These findings may thus have implications in individuals suffering from chronic inflammatory conditions. With these discoveries we therefore may now be able to dissect the relative contribution of the immune system as well as the CNS in sickness responses normally occurring in infections.

Pain is a central response element in inflammation

The sickness response is a syndrome of pathophysiological responses to injury or infection characterized by fever, increased sleep, and loss of appetite and water intake as well as lethargia. In this

condition everything seems and feels worse, because pain is facilitated, in association with hyperalgesia. In this issue, Watkins *et al.* summarize the current knowledge of how enhanced pain state can be the result of peripheral immune cells producing pro-inflammatory cytokines (TNF, IL-1 and IL-6) and how these molecules interact with the CNS [6]. Part of this pain response is due to direct CNS signalling of the systemically released cytokines (blood-born signal). However, of probably equal importance is the fact that these cytokines activate glia cells (astrocytes and microglia) present in high numbers in the spinal cord. Locally produced TNF or IL-1 targets the glia cells which then further have the capacity to produce additional pro-inflammatory cytokines [7]. Furthermore, unmyelinated sensory C-fibres, found in all major organs and tissues, store substance P and other pro-inflammatory tachykinins and released them in response to bacterial products, or tissue injury. This enhanced pain associated with the sickness response, also involves the sensory vagus nerve and the glosopharyngeal nerve and is a result of release of substance P, excitatory amino acids as well as nitric oxide. The net effect of all these signalling molecules is an increased neuronal excitability, increased Ca^{2+} influx and activation of *N*-methyl-D-aspertate receptors. Signals arising from nerve inflammation therefore activate a spinal cord–brain–spinal cord loop, which in turn activates glia [7].

Here, Watkins explains that the sensory or 'afferent' vagus nerve is intimately involved in inflammation, because it detects the presence of inflammation in the periphery and notifies the brain. It is the first step in the inflammatory reflex. Consider that vagotomy blunts development of fever in animals exposed to low intra-abdominal doses of IL-1 or endotoxin, because the brain does not receive the message that there is inflammation in the periphery. Neurones in the vagus nerve express IL-1 receptor and IL-1 as well as discrete IL-1-binding sites on so called glomus cells present in the paraganglia on the nerve. These data illustrate that pathological pain (induced by inflammation) is not simply a strict neuronal phenomenon, but is also a component of the immune response, and is modulated by peripheral immune cells and spinal cord glia cells. This may be of importance for future development of novel drugs for neuropathic pain as well as our understanding of increased risks for infections in anaesthetic skin areas. Regardless of how glia ultimately become activated, the implications for neuropathic pain treatment are clear. Neuropathic pain, regardless of whether it arises from traumatic or inflammatory insults, is essentially uncontrolled by currently available drug therapies. Thus, controlling pathological pain via targeting of spinal pro-inflammatory cytokines is an exciting possibility for clinical pain control.

Autoimmunity and sepsis morbidity – a reflection of autonomic nerve dysfunction in the inflammatory reflex?

A general view in medicine is that infections induce overproduction of cytokines that cause the clinical manifestations of this sickness response. This in turns activates 'the inflammatory reflex', a physiological pathway in which the autonomic nervous system detects and localizes the presence of the inflammatory stimuli [1]. Afferent signals to the brain including pain are then transmitted via the vagus nerve generating a reflex causing an anti-inflammatory response, which is partly mediated by the efferent branch of the vagus nerve. In this issue Tracey *et al.* outlines this pathway, and explains that the cholinergic anti-inflammatory pathway inhibits acute cytokine release [8]. The stimulated vagus nerve releases acetylcholine, which interacts with a specific receptor on macrophages in the reticuloendothelial system. This ligand receptor interaction leads to the specific inhibition of macrophage activation, and functions as a potent anti-inflammatory pathway. They provide evidence that this deactivation of macrophages occurs via acetylcholine binding to the alpha-7 subunit of the acetylcholine receptor [9]. These receptors are present on both macrophages and endothelial cells. Tracey *et al.* have thus identified a synapse between the cholinergic nervous system and innate immunity, which requires expression of acetylcholine binding to nicotinic acetylcholine receptors. Indeed they demonstrate that nicotine and electrical vagus nerve stimulation significantly inhibit the release of TNF as well as HMGB1 from endotoxin-stimulated macrophages [8]. HMGB1 is a 30-kDa nuclear and cytosolic protein widely studied as a transcription factor and growth factor that has recently been identified as a cytokine mediator of lethal systemic inflammation, arthritis and local inflammation [10–12]. In addition, they show that this anti-inflammatory pathway can inhibit adhesion

molecule expression on endotoxin-stimulated endothelial cells.

The identification of an immediate vagus-mediated regulation of pro-inflammatory cytokine production in tissue macrophages can have immense importance for development of novel anti-inflammatory therapeutic options. Indeed electrical vagus nerve stimulation currently in clinical use for epilepsy and depression as well as nicotine or selective alpha-7 acetylcholine receptor agonists may be launched. Tracey *et al.* provide evidence that activation of the alpha-7 acetylcholine receptor generates inhibition of NF-κ B signalling; however, it does not inhibit activation of several mitogen-associated protein kinases which are typically triggered via Toll-like receptor (TLR) interactions. As a consequence the efferent vagus nerve can restrict general inflammation in a fast, integrated and polarized fashion. However, it does not block induction of inflammation induced by TLR upon recognition of pathogen-associated molecular patterns (Microbial products) [8]. This novel anti-inflammatory aspect of the inflammatory reflex may be of great importance to unravel by developing agonists for the α-7 subunit receptor for potential clinical efficacy in sepsis. The potential of this pathway was also elucidated by a recent report by Wang *et al.*, demonstrating that treatment with the acetylcholine receptor agonist nicotine modulated the inflammatory system and reduced mortality in a mouse model of sepsis [13].

One may also speculate that dysfunction of this cholinergic anti-inflammatory pathway may play a central role in the occurrence of autoimmune diseases including diabetes, ulcerative colitis and rheumatoid arthritis, as impairment of this axis may result in systemic inflammation even at low microbial exposures.

I think that these papers represent a paradigm shift for medicine and immunology. The concept that the autonomic nervous system controls the immune system via a hard-wired network has widespread theoretical and practical implications. The theory that the immune system has been added to the list of other systems known to be controlled by the vagus nerve (cardiovascular, hepatobiliary and gastrointestinal) resonates with a sense of logic. It makes sense that the brain collects data about the status of the immune system, like a sixth sense, and acts to control the magnitude of the response, in real time, via its nerves. On the practical side, one can sense the importance for further study to control this pathway for therapeutic gain. Consider that higher cortical function, biofeedback, acupuncture, and meditation have all been implicated in altering vagus nerve signalling in humans; whether these responses influence immune function is unknown, but testable. In the future we may see clinical trials of experimental agents that influence this pathway to suppress inflammation, by targeting the specific receptors at critical control points. Finally, vagus nerve stimulators are already used in patients with epilepsy, and it now seems possible that their use can be tested for control of inflammatory diseases. It is an exciting time for research in this new field, and hopes are high that there may follow a practical benefit to future patients.

Conflict of interest statement

No conflict of interest was declared.

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