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DOI

[10.2119/molmed.2011.00065](https://doi.org/10.2119/molmed.2011.00065)

Publication date

2011

Document Version

Final published version

Published in

Molecular Medicine

[Link to publication](#)

Citation for published version (APA):

Koopman, F. A., Stoof, S. P., Straub, R. H., van Maanen, M. A., Vervoordeldonk, M. J., & Tak, P. P. (2011). Restoring the Balance of the Autonomic Nervous System as an Innovative Approach to the Treatment of Rheumatoid Arthritis. *Molecular Medicine*, 17(9-10), 937-948. <https://doi.org/10.2119/molmed.2011.00065>

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Restoring the Balance of the Autonomic Nervous System as an Innovative Approach to the Treatment of Rheumatoid Arthritis

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The immunomodulatory effect of the autonomic nervous system has raised considerable interest over the last decades. Studying the influence on the immune system and the role in inflammation of the sympathetic as well as the parasympathetic nervous system not only will increase our understanding of the mechanism of disease, but also could lead to the identification of potential new therapeutic targets for chronic immune-mediated inflammatory diseases, such as rheumatoid arthritis (RA). An imbalanced autonomic nervous system, with a reduced parasympathetic and increased sympathetic tone, has been a consistent finding in RA patients. Studies in animal models of arthritis have shown that influencing the sympathetic (via α - and β -adrenergic receptors) and the parasympathetic (via the nicotinic acetylcholine receptor $\alpha 7nAChR$ or by electrically stimulating the vagus nerve) nervous system can have a beneficial effect on inflammation markers and arthritis. The immunosuppressive effect of the parasympathetic nervous system appears less ambiguous than the immunomodulatory effect of the sympathetic nervous system, where activation can lead to increased or decreased inflammation depending on timing, doses and kind of adrenergic agent used. In this review we will discuss the current knowledge of the role of both the sympathetic (SNS) and parasympathetic nervous system (PNS) in inflammation with a special focus on the role in RA. In addition, potential antirheumatic strategies that could be developed by targeting these autonomic pathways are discussed.

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Online address: <http://www.molmed.org>

doi: 10.2119/molmed.2011.00065

INTRODUCTION

Rheumatoid arthritis (RA) is a common, immune-mediated inflammatory disease affecting about 1% of the adult population worldwide. RA is characterized by inflammation of the synovium leading to progressive destruction of cartilage and bone (1,2). Although its exact etiology currently remains unknown, advances in understanding the pathogenesis and underlying mechanisms have led to the development of new and more effective antirheumatic drugs. Despite these improvements, a

significant number of RA patients do not respond to the current therapies, and a need for identification of new pathways involved in modulation of inflammation to develop new antiinflammatory treatments still remains.

One such approach could be via manipulation of the autonomic nervous system. The nervous system is divided into the peripheral nervous system, with the sensory, somatic (voluntary) and autonomic (involuntary) sections, and the central nervous system. Classically, the autonomic nervous system is further di-

vided into the sympathetic (SNS) and parasympathetic nervous system (PNS), which are in tight equilibrium. These systems typically act in opposition to each other, but are able to function in synergy, making it difficult to predict the effects of autonomic nervous system activation. In general, stimulation of the SNS brings the body to a state of raised activity and attention, usually called the *fight or flight* response: heart rate and blood pressure increase, liver glycogen is converted into glucose and peristalsis of the gastrointestinal tract is temporarily inhibited. In contrast, stimulation of the PNS can be summarized as the *rest and digest* response, as this returns the body functions back to normal: blood pressure lowers, heart rate slows down, gastrointestinal peristalsis is turned on again and the liver starts producing new glycogen (3).

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Submitted February 17, 2011; Accepted for publication May 19, 2011; Epub (www.molmed.org) ahead of print May 20, 2011.

It has become apparent that the nervous system has multiple anatomical and physiological connections with the immune system. Through these pathways, the nervous and immune systems have extensive communications using neurotransmitters, cytokines and endocrine hormones (4). Consequently, the nervous system is able to detect and regulate inflammation in peripheral tissues and is involved in maintaining immune homeostasis. An overview of the role of the autonomic nervous system in inflammation is shown in Figure 1.

Several observations have supported the notion that there may be an interaction between the nervous system and inflammation in RA. For instance, it has been reported that hemiplegic patients who develop RA do not develop arthritis on their hemiplegic (denervated) side (5). This eventually led to the hypothesis that experimental modulation of the body's neural pathways involved in regulating inflammation could potentially lead to the development of new treatment options for different inflammatory diseases, including RA.

SYMPATHETIC NERVOUS SYSTEM

Functional Anatomy of the Sympathetic Nervous System

The autonomic nervous system runs from the central nervous system to the peripheral organs through two different neurons: the *preganglionic* and the *postganglionic neurons*. The cell body of a preganglionic neuron is located in the central nervous system, between the first thoracic (Th1) and third lumbar (L3) spinal cord segments, from where its axon connects to the postganglionic neuron. Subsequently, the axon of the postganglionic neuron projects to the target organ (6).

All regions of the body receive sympathetic innervation. This enables the SNS, together with the hypothalamic-pituitary-adrenal (HPA) axis, to be a key peripheral regulator in maintaining internal homeostasis. Although discussion of the HPA axis is beyond the scope of

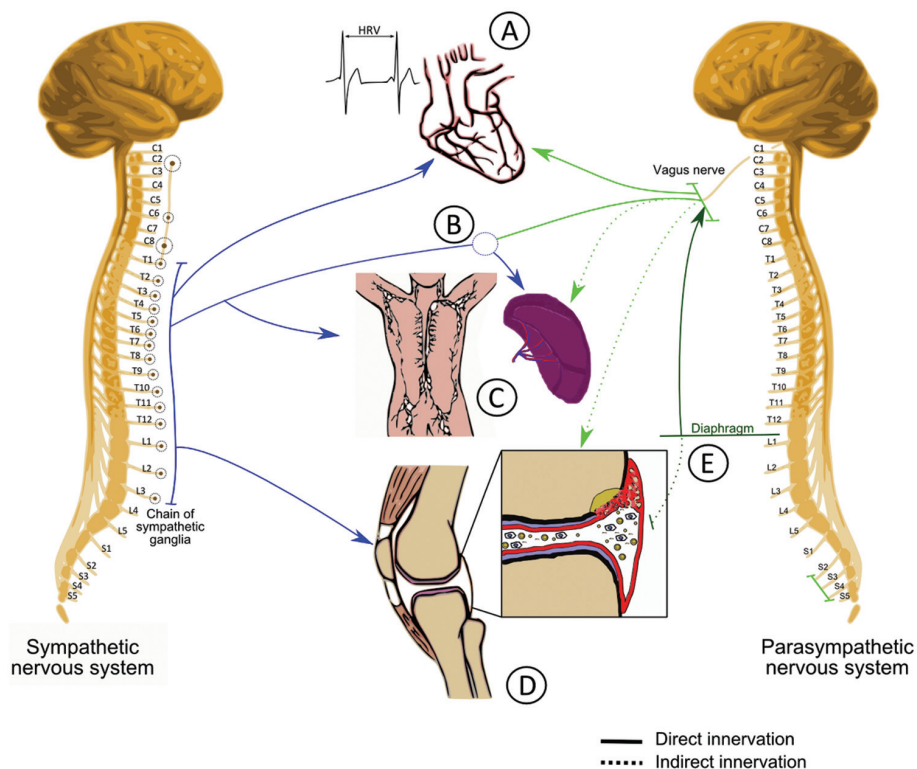


Figure 1. The role of the autonomic nerve system in rheumatoid arthritis. The autonomic nervous system is divided into the sympathetic (left) and parasympathetic (right) nervous system. Cell bodies of preganglionic sympathetic neurons are located in the central nervous system, between the first thoracic (Th1) and third lumbar (L3) spinal cord segments, from where its axon connects to the postganglionic neurons. Parasympathetic preganglionic neurons are found in the brainstem and the second through fourth sacral (S2–S4) spinal cord segments. The parasympathetic nervous system mainly consists of the vagus nerve, the tenth cranial nerve. The heart (A) is innervated by both the sympathetic and parasympathetic nervous system. Heart rate variability (HRV) is a technique to determine the balance of the autonomic nervous system. In rheumatoid arthritis patients, this balance can be altered compared with healthy individuals. This imbalance is caused by a dominant influence of the sympathetic nervous system instead of the parasympathetic nervous system, which under normal conditions, to a great extent, determines HRV. Both lymphoid organs (spleen and lymph nodes) (C) and joints (D) are innervated directly by the sympathetic nervous system, but no innervation of the vagus nerve (parasympathetic nervous system) has been found. Stimulating the vagus nerve and thereby reducing inflammation (a concept known as the *cholinergic antiinflammatory pathway*) does, however, need the presence of the spleen. Fibers of the vagus nerve end in the celiac-superior mesenteric plexus (B), further innervation of the spleen via the splenic nerve has been found to contain only catecholaminergic fibers from the sympathetic nervous system. It is not known how the vagus nerve can signal via the spleen without direct innervation, but it is proposed that the catecholaminergic splenic nerve transmits the parasympathetic signal toward the spleen. Inflammation is registered in the brain via the circulation, but also via the afferent vagus nerve (E). Subdiaphragmatic vagotomy inhibits signaling of inflammation toward the brain. As the joint is not innervated by the vagus nerve, most likely direct detection of joint inflammation is not possible.

this review, it must be mentioned that this axis and the SNS are involved in a mutual, positive feedback loop and acti-

vation of one system usually activates the other as well (7). When homeostasis is disturbed, both the HPA axis and the

SNS become activated to restore the internal milieu.

Catecholamines, such as epinephrine (adrenaline), norepinephrine (noradrenaline, NE) and dopamine, are released by the adrenal gland or nerve tissue and act as sympathomimetic hormones or neurotransmitters. In response to a stimulus, most sympathetic postganglionic neurons release NE, which subsequently activates adrenergic receptors (ARs) on the peripheral target tissue. There are two adrenergic receptor types (α - and β -AR), and both exhibit a different affinity for NE, depending on the receptor subtype (α_1 , α_2 , α_3 , β_1 or β_2) and the tissue where they are expressed (8). The effect of NE on the different receptors also depends on the receptor's local concentration in the synaptic cleft: a high NE concentration (1–100 $\mu\text{mol/L}$) acts on both α - and β -ARs, but at low concentrations ($\leq 0.1 \mu\text{mol/L}$), NE predominantly stimulates α -ARs (9).

General Role of Sympathetic Nervous System in Inflammation

The regulatory function of the SNS includes monitoring and influencing immune homeostasis. During an immune response, peripherally secreted proinflammatory cytokines, such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)- α , can signal to the brain in two ways: via the circulation (4) or through afferent fibers of the vagus nerve (10,11) (also see Role of Vagus Nerve: Cholinergic Antiinflammatory Pathway below). This can result in central activation of the SNS, which, in turn, stimulates catecholamine production in the different lymphoid organs via the efferent, sympathetic fibers (4,7).

Both primary (bone marrow, thymus) and secondary (spleen, lymph nodes, mucosa-associated lymphoid tissue) lymphoid organs are innervated extensively by the SNS (7,8,12). Interestingly, the distribution and density of sympathetic nerves in lymphoid organs are not stable, but appear to change in response to alterations in the microenvironment, including immune activation and changes

in cytokine release (12–14). The immune system is thereby capable of influencing the SNS and the innervation of lymphoid organs allows the SNS to influence the immune cells directly, via released NE which binds to ARs on these cells.

Cells of the adaptive immune system primarily express the β_2 -subtype, whereas cells of the innate immune system appear to express not only β_2 -, but also α_1 - and α_2 -ARs (8,12). Stimulation of these subtype receptors on macrophages can elicit different functional effects. Macrophages can be activated through α_2 -AR stimulation, while stimulation of the β_2 -AR has a suppressive effect on macrophage activity (12). For example, during late-stage sepsis, β_2 -ARs were upregulated on rat liver Kupffer cells, a macrophage subset, leading to inhibition of Kupffer cell function and subsequent immunosuppression (15). Thus, not only the density of SNS fibers, but also the AR binding capacity on immune cells, is adaptive to environmental needs. In addition, immune function is regulated by peptide neurotransmitters that colocalize with NE, such as neuropeptide Y and adenosine triphosphate (ATP), as well, but their exact contribution to immune regulation remains to be unraveled (12).

The role of SNS activation in immune cell functioning is complex. *In vitro* and *in vivo* studies on the effects of NE, or different adrenergic agonists and antagonists on immune cells report dissimilar results (8,12). In short, NE's effects during an immune reaction appear to be dependent on which immune cells are present, the activation state of these cells, the type and number of ARs they express (see above), and the surrounding cytokine context. Most evidence points toward the fact that SNS activity is able to inhibit the development of a T helper (Th)1 immune response and shift the Th1/Th2 balance toward a Th2 immune response (7,12). Besides influence on proliferation of immune cells and their cytokine production, the SNS also appears to be involved in regulation of leukocyte trafficking to sites of in-

flammation (16). The exact mechanism by which the SNS or adrenergic agents influence cell migration is as yet unknown, but this might be due to the sympathetic influence on vascular smooth muscle regulating the blood flow and thereby the lymphocyte delivery (8). It also has been shown that stress leads to changes in expression of cell adhesion molecules on immune cells (17,18), thereby possibly influencing cell migration from the blood to the site of inflammation.

Sympathetic Nervous System and Arthritis

In general, RA patients have an autonomic imbalance with an overly active SNS and reduced PNS activity (19). Determining the heart rate variability with an electrocardiogram is an established technique to evaluate this autonomic balance. A low variability in heart rate reflects an increase in SNS activity and reduced PNS activity (Figure 1A). In established RA, it has been suggested that stress may aggravate disease activity. Minor stress (lasting from minutes to a few days with small intensity), that is, *daily hassles*, may lead to a short rise of neurotransmitters and hormones, and could increase disease activity in RA patients (20). The role of the stress response is however far from clear. The Lewis rat, for instance, has a defective stress response system and is much more sensitive to the development of arthritis than F344 rats, which are characterized by a hyperactive SNS (21).

β_2 -Adrenergic Receptor Expression and Function in Rheumatoid Arthritis

β_2 -ARs have been shown to have a role in the time-dependent, immunomodulating effect of the SNS (22–24) and they are expressed on innate and adaptive immune cells of humans and rodents (7). Peripheral blood mononuclear cells (PBMCs), specifically monocytes, B cells and CD8⁺ T cells, from RA patients express lower levels of β_2 -ARs compared with healthy subjects (25) and the receptor density on RA synovial

fluid lymphocytes is even less (26). RA PBMCs therefore also are less responsive to NE treatment compared with healthy subjects: upon NE administration *in vitro* (25,27) they are less effective in suppressing T-cell activation and proliferation, and the stimulated cytokine response was not decreased as much. In active systemic and polyarticular juvenile RA there is a diminished cAMP production upon β 2-AR stimulation, indicating a defective neuroimmune response (28).

α -Adrenergic Receptor Expression and Function in Rheumatoid Arthritis

The role of α -AR subtypes in arthritis is less clear. As mentioned above, only cells from the innate immune system appear to express α -ARs (8,12). Normally, α 1-ARs are expressed only on natural killer cells, whereas α 2-ARs are present on natural killer cells, monocytes and macrophages (12). However, *in vitro* studies showed that treating normal monocytes with β 2-AR agonists can induce expression of the α 1-AR subtype (29). On the contrary, in RA patients with high disease activity, catecholamines mainly mediate their effect through α 1-ARs on PBMCs (30,31), while showing a decreased density of β 2-ARs (25,31). Functional α 1-ARs also appeared to be upregulated on leukocytes of juvenile idiopathic arthritis patients, while being absent on leukocytes from healthy donors, and stimulation of these receptors induced higher IL-6 levels (32). Furthermore, stimulation of α 2-ARs can initiate proliferation of fibroblast-like synoviocytes (FLS) in the joint (12,30), which will lead to increased production of cytokines, proteases and chemokines. Subsequently, more immune cells may be recruited to the joint, eventually resulting in the cartilage destruction typically seen in RA. It has been suggested that α -ARs might become more relevant in a later stage of chronic inflammation, coinciding with decreased numbers of β -ARs. This observation has been called the β -to- α -adrenergic shift (30).

Immunomodulatory Effect of Agents Targeting the Sympathetic Nervous System in Animal Models of Arthritis

Intervening with SNS activity may influence the inflammatory process seen in RA, either locally or systemically. To date, clinical testing of AR agents as a treatment option for RA patients has not been performed and our current knowledge is based on data obtained in animal models of arthritis. In the sections below, the results of these studies are discussed and in Table 1 the available experiments are summarized.

Time-dependent effect of adrenergic intervention. Studies with AR drugs in animal models of arthritis showed conflicting results: both exacerbating and ameliorating effects of AR agonists on arthritis severity were seen (22,23,33,34). This might be due to the timing of drug administration, and it is now well established that the SNS induces time-dependent, immunomodulating effects in arthritis models: prior to arthritis induction and in the asymptomatic phase, SNS activation has a proinflammatory effect, whereas, after onset of arthritis, SNS activation may inhibit inflammation (24,30,35). This was nicely shown in a collagen-induced arthritis (CIA) mouse model in mice in which chemical sympathectomy (leading to a 75% reduction in NE production) was initiated by intraperitoneal (i.p.) injections with 6-hydroxydopamine (6-OHDA) at different time points (35) (see also Table 1). The transition phase from pro- to anti-inflammatory effects by the SNS is thought to lie between day 30 and day 55 after immunization in murine CIA (14,35). It has been suggested that the early, proinflammatory effects are caused by SNS-induced redistribution, migration and chemotaxis of leukocytes toward the site of inflammation (30,35). Interestingly, the influence of the SNS on CD4⁺CD25⁺ T cells seems to have a role in arthritis severity. SNS intact, splenectomized (to minimize endogenous CD4⁺ T cells), recipient CIA mice received either CD4⁺CD25⁺ T cells from SNS-intact CIA mice or from sympathectomized CIA

mice. The CD4⁺CD25⁺ T cells that were influenced by an intact SNS promoted arthritis compared with the SNS-deprived CD4⁺CD25⁺ T cells. Thus, it appears likely that the SNS has an effect on the FoxP3⁻ subset of proinflammatory CD4⁺CD25⁺ T cells. The CD4⁺CD25⁺ T cells from the sympathectomized CIA mice also displayed a reduced cytokine response (36).

β 2-Adrenergic receptor agonists or antagonists in experimental arthritis. To our knowledge, no studies on the effect of β 1-agonists on experimental arthritis have been performed. β 2-ARs presumably play an important role in the time-dependent, immunomodulating effect of the SNS. In studies that used the adjuvant-induced arthritis (AIA) model in rats, treatment with β 2-AR agonists resulted in worsening of disease severity, when this was initiated prior to or at the time of adjuvant challenge (22,24), while administration of β 2-AR antagonists started prior to adjuvant challenge significantly reduced the severity of joint injury and delayed disease onset (22,23). Furthermore, initiating β 2-AR agonist treatment at or after onset of disease also led to a reduction in disease severity (24) (Table 1).

α -Adrenergic receptor agonists or antagonists in experimental arthritis. *In vivo* studies on the function of α -AR subtypes in experimental arthritis have shown opposing results. Blocking with a nonspecific antagonist of α -AR (both α 1- and/or α 2-AR) has a different effect on disease severity depending on the time that the agent is given (23,24). If administered at disease onset, it reduces the severity of disease, but aggravation of disease severity is observed when given prior to immunization (24). Specific blocking of the α 1-AR with an antagonist prior to disease onset did not result in an effect on experimental arthritis (23,33), but using an α 2-AR antagonist does increase arthritis severity in experimental arthritis in Sprague-Dawley rats (33). The aggravation of arthritis when treated with a nonspecific α -AR antagonist, when given before immunization, might,

Table 1. Overview of studies describing the role of the sympathetic nervous system using adrenergic receptor drug intervention in experimental animal models of rheumatoid arthritis.

Effect on SNS	Intervention	Arthritis model	Time point start intervention	Effect on arthritis	Authors
Single interventions					
Catecholamine depletion	ADMX ^a	AIA (Sprague-Dawley rats)	Prior to immunization	Decreased severity	(22)
	Systemic sympathectomy (6-OHDA)	CIA (DBA/1 mice)	Prior to immunization	Reduced severity	(35)
	Systemic sympathectomy (6-OHDA)	CIA (DBA/1 mice)	After disease onset, during active disease	Increased severity	(35)
	Reserpine	AIA (Sprague-Dawley rats)	Prior to immunization	Reduced severity + delayed onset	(23)
	Reserpine	AIA (Sprague-Dawley rats)	At disease onset	Reduced severity	(23)
	Reserpine (intraarticular injection)	CIA (DBA/1 mice)	After disease onset, during active disease	Reduced severity in injected joint	(41)
α 1-AR antagonist	Prazosin	AIA (Sprague-Dawley rats)	Prior to immunization	No effect	(22,33)
α 2-AR agonist	Clonidine	AIA (Sprague-Dawley rats)	Prior to immunization	Reduced severity	(33)
	NE (high dose)	AIA (Sprague-Dawley rats)	Prior to immunization	Reduced severity	(33)
α 2-AR antagonist	Yohimbine	AIA (Sprague-Dawley rats)	Prior to immunization	No effect	(23)
	Yohimbine	AIA (Sprague-Dawley rats)	Prior to immunization	Increased severity	(33)
α -AR antagonist	Phentolamine	AIA (Lewis rats)	At time of immunization	Increased severity	(24)
		AIA (Lewis rats)	At disease onset	Reduced severity	(24)
β 1-AR antagonist	Phenoxybenzamine	AIA (Sprague-Dawley rats)	Prior to immunization	No effect	(23)
	Metoprolol	AIA (Sprague-Dawley rats)	Prior to immunization	No effect	(23)
β 2-AR agonist	Terbutaline	AIA (Lewis rats)	At time of immunization	Increased severity	(24)
		AIA (Lewis rats)	At disease onset	Reduced severity	(24)
		CIA (DBA/1 mice)	After disease onset, during active arthritis	Reduced severity	(34)
β 2-AR antagonists	Butoxamine or ICI 118,551	AIA (Sprague-Dawley rats)	Prior to immunization	No effect	(33)
		AIA (Sprague-Dawley rats)	Prior to immunization	Reduced severity + delayed onset	(23)
		AIA (Sprague-Dawley rats)	At disease onset	Reduced severity	(23)
β 1/ β 2-AR agonist	Isoproterenol	AIA (Sprague-Dawley rats)	Prior to immunization	No effect	(33)
		AIA (Sprague-Dawley rats)	Prior to immunization	Reduced severity	(23)
β -AR antagonist	Propranolol	AIA (Sprague-Dawley rats)	Prior to immunization	Reduced severity	(23)
Combined interventions					
α - and β -AR agonists + α 1-AR antagonist	NE and prazosin	AIA (Lewis rats)	At disease onset	Reduced severity (NE effect)	(33)
α - and β -AR agonists + α 2-AR antagonist	NE and yohimbine	AIA (Lewis rats)	At disease onset	Increased severity (combined effect)	(33)
α - and β -AR agonists + β 1-antagonist	NE and metoprolol	AIA (Lewis rats)	At disease onset	Reduced severity (NE effect)	(33)
α - and β -AR agonists + β 2-AR antagonist	NE and butoxamine	AIA (Lewis rats)	At disease onset	Reduced severity (NE effect)	(33)
Catecholamine depletion + administration (α -AR and β -AR agonists)	ADMX and NE	AIA (Sprague-Dawley rats)	Prior to immunization	Increased severity compared with ADMX	(22)
Catecholamine depletion + β 2-AR agonist	ADMX and salbutamol	AIA (Sprague-Dawley rats)	Prior to immunization	Increased severity compared with ADMX	(22)

^aADMX = adrenal medullectomy.

therefore, be the result of an effect via the α_2 -AR (23,24).

Treatment with specific α_2 -AR agonists, given before disease onset, has a positive effect on arthritis (33). Nothing is known about the effect of specific α_1 -AR agonists on arthritis activity in animal models.

Sympathetic Innervation of the Spleen and Joints in Experimental and Rheumatoid Arthritis

Nervous innervation of the rodent and human spleen currently is believed to be primarily by SNS fibers, as no cholinergic fibers have been identified here so far (8,37) (Figure 1C). In spleens of arthritic rats (AIA), the nerve density in white pulp distal to the hilus is reduced considerably, while nerve density in the hilus and red pulp is increased (38). The red pulp, the site where activated immune cells reside and eventually exit the spleen (12), is normally sparsely innervated by sympathetic fibers, but a sympathectomy resulted in a compensatory sprouting of noradrenergic nerves in red pulp of arthritic, but not of nonarthritic rats (38). The investigators suggested that this altered sympathetic innervation pattern in the red pulp was thought to reflect a regulated microenvironment, where migrated immune cells provide trophic support to the redistributed SNS fibers, which, in turn, could play a critical role in sustaining immune dysregulation seen in chronic inflammatory stages of arthritis (38). In addition, an overall reduction of sympathetic nerve fibers was found in the spleen of DBA/1 mice with early, symptomatic CIA compared with normal DBA/1 mice. Despite this reduction, the SNS was still able to stimulate interferon- γ and cytokine-induced neutrophil chemoattractant-1 (CXCL-1) secretion by splenic T cells via various neurotransmitter systems (14). This demonstrates that the SNS can still contribute to disease-aggravating effects in the early phase of arthritis despite reduced fiber density (14). As far as we are aware, there are no data on altered sympathetic innervation density of the spleen in RA patients.

Sympathetic innervation of the joint is decreased largely in experimental arthritis, as was shown during the symptomatic stages of disease in murine CIA (39). Similarly, the number of sympathetic nerve fibers in the joints of RA patients is reduced significantly, coinciding with an increased degree of inflammation, but also with significantly more substance P-positive nerve fibers and an augmented NE production by synovial cells (macrophages, B cells, fibroblasts, mast cells and granulocytes) (9,40,41). Moreover, in AIA rats, ankle joints that developed more severe arthritis were more densely innervated by substance P-containing, primary afferent neurons than were joints that developed less severe arthritis (knees) (42). Infusion of substance P into these knees did increase arthritis severity, suggesting that this neuropeptide also may contribute to inflammation in RA (9,42). This implies a diminished influence of the SNS on, and more involvement of sensory inputs in, established joint inflammation.

In longstanding RA, a marked loss of sympathetic nerve fibers can be found (9). After reduction of sympathetic innervation in the inflamed joint, the synovial tissue itself has a role in producing NE (9,40). The concentration of NE in synovial tissue is in the α -adrenergic region 10^{-9} to 10^{-8} mol/L, which is known mainly to have an activating effect on the α -ARs. Moreover, in patients with RA, who markedly lose sympathetic nerve fibers, the density of catecholamine-producing cells was higher than in patients with OA, who do not lose sympathetic innervation. Blockade of catecholamine storage into vesicles by reserpine caused a very strong and specific inhibition of TNF- α mRNA and TNF- α protein from cells of patients with OA and RA (41). In addition, the effect of cytoplasmic catecholamine increase was tested *in vivo* in CIA in mice. It was found that local treatment with reserpine markedly reduced inflammation without causing systemic side effects in the animals (41). This indicates that when sympathetic

nerve fibers are lost in inflamed tissue, *sympathetic cells* can take over. These findings demonstrate that peripheral cells start producing catecholamines during chronic inflammation, and the increase of cytoplasmic catecholamines has strong anti-inflammatory effects *in vitro* and *in vivo*. Therefore, modulation of catecholamine-producing cells could be used as a new therapeutic target in arthritis.

PARASYMPATHETIC NERVOUS SYSTEM

Functional Anatomy of the Parasympathetic Nervous System

As mentioned in the introduction, activity of the PNS results in the *rest and digest* effect, enabling the body to restore its energy supplies. Parasympathetic, *preganglionic neurons* are found in the brainstem and in the second through fourth sacral (S2–S4) spinal cord segments (see Figure 1). The *postganglionic neurons* reside in parasympathetic ganglia on or near the target organs, in contrast to the sympathetic ganglia, which are located at greater distance from the target organs (6). The PNS consists mainly (75%) of the vagus nerve, the tenth cranial nerve (3), which is the largest nerve and owes its name to the wandering course it runs along the body (43). The efferent fibers of the vagus nerve originate mainly in the dorsal motor nucleus of the vagus and the nucleus ambiguus in the medulla oblongata (brainstem). From there, they pass through the neck and thorax to the abdomen, meanwhile branching off fibers to various organs, including the heart, lungs, gastrointestinal tract and pancreas (43,44).

Acetylcholine (ACh) is the main parasympathetic, postganglionic neurotransmitter, but it also is released in all preganglionic neurons, both parasympathetic and sympathetic. The enzyme choline acetyltransferase (ChAT) synthesizes ACh from choline and acetyl-CoA, and acetylcholinesterase (AChE) can degrade it into the inactive metabolites choline and acetate. ACh can bind to two types of receptors, the muscarinic (mAChR) and the nicotinic (nAChR) re-

ceptors, which are both widely expressed in neuronal as well as nonneuronal cells and can consist of different subunits. Many different nicotinic ($\alpha 1$ – $\alpha 10$ and $\beta 1$ – $\beta 4$) and muscarinic (M1–M5) subunits have been identified, leading to a wide variety of possible receptors with different physiological functions (45–47). In the context of rheumatoid and experimental arthritis not much is known about the role of various nicotinic ($\alpha 1$ – $\alpha 6$, $\alpha 8$ – $\alpha 10$) and muscarinic (M1–M5) receptors. Therefore, in this review we will focus specifically on the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR).

The nonneuronal cells that can produce ACh, for example, epithelial, endothelial, mesothelial, muscle, and immune cells, also possess all functional components of the cholinergic system (48) and the cholinergic signaling in these cells is comparable to regular neurotransmission (49). Furthermore, it has been suggested that dysfunction in these nonneuronal cholinergic systems might be involved in the pathogenesis of several chronic diseases, such as colitis ulcerosa, cystic fibrosis and psoriasis (49).

General Role of Parasympathetic Nervous System in Inflammation

How exactly the PNS and, in particular, the vagus nerve are involved in the immune system is still under investigation. A summarizing review from 2007 that covered 20 years of neuroanatomical research on the autonomic innervation of the immune system stated that there is no neuroanatomical evidence for parasympathetic or vagal nerve innervation of any immune organs (8,50) (see Figure 1C). This conclusion was based on numerous studies that reported negative results for retrograde tracing from the immune organs to vagal brainstem nuclei, combined with absence of ACh, vesicular ACh transporter-labeled fibers and/or ACh-metabolizing enzymes, such as ChAT and AChE, in the immune organs. In addition, studies using electrical field stimulation of spleen slices indicated that sympathetic, but not cholinergic, nerve fibers innervate the spleen.

(14). On the other hand, there was local ACh expression, but this could be explained theoretically by release from splenic immune cells. In spite of the apparent absence of direct vagal nerve innervation, however, there is evidence that the spleen receives not only sympathetic signals, but also parasympathetic input (51). Recent work has shown that the vagus nerve may inhibit TNF- α production by splenic macrophages via a signal from the celiac-superior mesenteric plexus projecting in the splenic nerve, which is comprised principally of catecholaminergic fibers (Figure 1B) (see section Route of the Cholinergic Antiinflammatory Pathway below) (52).

Role of Vagus Nerve: Cholinergic Antiinflammatory Pathway

Whether directly innervating immune organs or not, it has been widely acknowledged that the PNS, through the vagus nerve, plays an important role in regulation of inflammation. Stimulation of peripheral, *afferent* vagus nerve fibers by endotoxin or cytokines can activate the HPA axis and SNS centrally, resulting in peripheral release of antiinflammatory glucocorticoids and NE (7,53–57). In addition, it has been shown that *efferent* vagus nerve fibers are involved in inflammatory modulation, by performing bilateral cervical vagotomy in a rat model of experimental sepsis (58). Subsequent electrical stimulation of the peripheral part of the vagus nerve significantly decreased serum TNF- α levels and prevented development of shock compared with vagotomized rats that did not receive electrical stimulation. This peripheral vagus nerve stimulation (VNS) in the vagotomized animals was not followed by enhanced glucocorticoid production, indicating that the suppressed TNF- α production could not be attributed to activation of the HPA axis, but was achieved merely through the efferent vagus nerve (58). This has been confirmed in subsequent studies, where VNS significantly attenuated TNF- α synthesis and improved clinical outcome in experimental models

of hemorrhagic shock (59,60) and ischemia-reperfusion injury (59,60), and blocked leukocyte migration by inhibiting endothelial cell activation, a key regulator of leukocyte trafficking during inflammation, in the carrageenan air pouch rat model (61). This antiinflammatory influence of the efferent vagus nerve is now well known as the *cholinergic antiinflammatory pathway* (62).

The $\alpha 7$ Nicotinic Acetylcholine Receptor

In a first attempt to determine how the efferent vagus nerve establishes its antiinflammatory effect, *in vitro* experiments studying the cytokine release by primary human macrophages showed that ACh dose-dependently inhibited release of TNF- α , IL-1 β and IL-6 after 4 and 20 h of LPS stimulation, primarily through nAChRs; production of the antiinflammatory cytokine IL-10 was unaffected (58). Nicotinic AChRs, which are integral membrane proteins belonging to the ligand-gated, ion channel superfamily, are further subdivided into muscle-type nicotinic receptors, found at the neuromuscular junction, and neuronal-type nicotinic receptors. In humans, 17 different subunits have been identified ($\alpha 1$ – $\alpha 10$, $\beta 1$ – $\beta 4$, γ , δ , ϵ) and five transmembrane subunits are assembled around a central pore to form a homo- or heteromeric nAChR (63). Subsequent research led to the identification of the neuronal-type $\alpha 7$ nACh receptor subtype ($\alpha 7$ nAChR) as the essential regulator of the antiinflammatory effect of ACh (64). Additional *in vitro* studies using human monocytes, PBMCs and whole blood showed that nicotine and the $\alpha 7$ nAChR selective partial agonist GTS-21 dose-dependently inhibited the release of TNF- α and IL-1 β after LPS stimulation, with GTS-21 being more potent than nicotine (64–66).

The $\alpha 7$ nAChR consists of five $\alpha 7$ subunits that assemble to form a ligand-binding, homopentameric ion channel (67). The receptor is found not only on neurons and macrophages, but also is expressed widely on other nonneuronal cells, including immune cells, such as

monocytes, T and B lymphocytes, dendritic cells (68) and RA FLS (69–71). In addition to this classical variant of the $\alpha 7$ receptor, the human genome contains an alternative, partial duplication of the $\alpha 7$ subunit gene, called the *dupa7* or “cholinergic receptor family with sequence similarity to $\alpha 7$.” The main difference between this and the classical $\alpha 7$ is that this *dupa7* subunit lacks binding sites for nicotine or other $\alpha 7$ agonists/antagonists and, to date, the role of the *dupa7* remains unclear. In fact, although it is transcribed in different cells, including human leukocytes (72) and FLS (69), it is not clear whether this transcript is ever translated into a functional protein and eventually can form a functional receptor (68,72). It is possible that the *dupa7* has a regulatory role, intervening or contributing to the function of the classical $\alpha 7$ nAChR and therefore might provide new pharmacological options (69).

Several *in vivo* studies confirmed the importance of the $\alpha 7$ nAChR in the cholinergic antiinflammatory pathway. Receptor activation by specific agonists effectively attenuated immune responses and ameliorated disease severity in different experimental settings, including animal models for sepsis (73,74), pancreatitis (75), ischemia-reperfusion injury (76), postoperative ileus (77), acute lung injury (73), and, as discussed in more detail below, CIA (78,79). Moreover, in $\alpha 7$ -subunit-deficient mice, VNS failed to reduce serum TNF- α levels during endotoxemia (64).

Route of the Cholinergic Antiinflammatory Pathway

Not only the $\alpha 7$ nAChR is essential in the cholinergic antiinflammatory pathway, the spleen is thought to play an important role as well in the regulation of systemic inflammation by the efferent vagus nerve, since electrical VNS failed to diminish serum TNF- α levels in splenectomized mice treated with endotoxin (80). As mentioned earlier, there is no convincing evidence that the spleen is actually innervated by the vagus nerve (see Figure 1B). In this light, a further

study on unraveling the road of the cholinergic antiinflammatory pathway revealed that the nerve fibers found in apposition to the TNF- α -secreting macrophages in the spleen are catecholaminergic and not cholinergic (52). These catecholaminergic nerves proved to be required for functional inhibition of TNF- α production by VNS. It is thought that the vagus nerve functionally communicates with the splenic nerve at the level of the celiac and superior mesenteric ganglia which may lead to secretion of catecholamines, such as NE, by the splenic nerve (52). NE has been shown to have a direct, inhibitory effect on TNF- α secretion by splenic macrophages by acting on ARs (81,82). In addition, there is a direct inhibitory effect of ACh on TNF- α secretion by macrophages through activation of the $\alpha 7$ nAChR (64). As the spleen contains and releases ACh upon splenic nerve stimulation (83,84), ACh is most likely produced by immune cells present in the spleen, which (also) attenuates cytokine production by signaling through $\alpha 7$ nAChR on TNF- α -producing cells (52).

Interestingly, it was shown recently that GTS-21 can reduce serum TNF- α levels in both normal and splenectomized animals using an animal model of hemorrhagic shock (85). It is, therefore, conceivable that the cholinergic antiinflammatory pathway consists of two separate arms: (i) the inhibitory effect of ACh produced by nonneuronal cells on cytokine production by immune cells at the site of inflammation through $\alpha 7$ nAChR activation and (ii) the immunosuppressive effect of the efferent vagus nerve by communicating with the splenic nerve via the celiac and superior mesenteric ganglia, and the subsequent stimulation of both $\alpha 7$ nAChRs and ARs in the spleen.

Parasympathetic Nervous System in Experimental and Rheumatoid Arthritis

We have described recently the role of the cholinergic antiinflammatory pathway in murine CIA (78). Systemic treatment with nicotine or the $\alpha 7$ nAChR spe-

cific agonist AR-R17779 significantly ameliorated arthritis activity, whereas unilateral cervical vagotomy exacerbated the disease. The effect of AR-R17779 appeared more potent than that of nicotine, pointing to an important role of the $\alpha 7$ nAChR in mediating the antiinflammatory effect. Moreover, AR-R17779 hardly crosses the blood-brain barrier, indicating that these effects are achieved by stimulation of peripheral $\alpha 7$ nAChRs. We also tested the pharmacological and functional profile of two novel compounds, CTI-15311 and CTI-15072, with different effects on ion channel activity and investigated their role in modulating CIA (unpublished results, van Maanen *et al.*). Although both compounds bound to $\alpha 7$ nAChR with high affinity, CTI-15311 acted like a classical agonist of ion channel activity, whereas CTI-15072 did not produce an electrical current, suggesting it can act as an ion channel antagonist. Despite not being able to induce significant levels of ion channel activity, CTI-15072 could still improve arthritis, albeit to a lesser extent than CTI-15311. Moreover, CTI-15072 was clearly distinct from typical competitive antagonists, since it was able to synergize with the positive allosteric $\alpha 7$ nAChR-modulator PNU-120596 to induce detectable ion channel current, suggesting that it is a selective desensitizer of $\alpha 7$ nAChR. These data provide direct evidence that the $\alpha 7$ nAChR on immune cells does not require typical ion channel activation to exert its antiinflammatory effects. Moreover, CTI-15072 only showed 6% brain penetration, indicating that the antiinflammatory effect on experimental arthritis via the $\alpha 7$ nAChR can be observed despite limited penetration of the central nervous system.

In subsequent experiments, it was demonstrated that clinical arthritis scores and synovial inflammation were increased markedly in $\alpha 7$ nAChR-deficient mice compared with their wild-type littermates in both the acute and chronic phase of the disease (86). These data suggest that the $\alpha 7$ nAChR, and thereby the cholinergic antiinflammatory pathway, is

involved in the regulation of arthritis activity in experimental arthritis. More evidence pointing toward a role for the $\alpha 7$ nAChR in arthritis was obtained from experiments using whole blood from RA patients, who exhibited suppressed vagus nerve activity (87). Upon stimulation, inflammatory cells from these whole blood cultures produced less TNF- α as compared with healthy controls, but the addition of cholinergic agonists to the stimulated whole blood cultures still suppressed cytokine production significantly, implying that targeting the $\alpha 7$ nAChR could indeed be a new treatment option in these patients (87).

Presence of the $\alpha 7$ Nicotinic Acetylcholine Receptor in the Joint

To date, there is no explicit evidence of vagal innervation of the joints (Figure 1D). However, we and others have shown that the $\alpha 7$ nAChR is expressed in synovial tissue and on RA FLS. *In vitro* stimulation of the receptor (with ACh or $\alpha 7$ nAChR agonists) suppressed TNF- α and IL-1 β -induced production of IL-6 and IL-8 by RA FLS (69,70). Furthermore, the presence of a nonneuronal cholinergic system in human synovial tissue was described recently. Synovial biopsies from RA and OA patients showed expression of ChAT in both mononuclear-like cells as well as FLS, indicating that ACh can be produced locally by these cells (88). This local ACh production might be important in the regulation of joint inflammation, thereby contributing to the cholinergic antiinflammatory pathway. Whether the release of nonneuronal ACh is triggered by neuronally released ACh or if the nonneuronal cholinergic system in synovial tissue acts independently from cholinergic nerves remains to be investigated further (89).

The regulation of immune function is certainly not bound to be exerted by ACh alone, but a potentiating effect of other endogenous cholinergic agonists may be conducive. One such agonist is SLURP-1 (secreted mammalian Ly6/urokinase plasminogen-type activator receptor-related protein-1), which

is produced by both lymphocytes and macrophages, and has been shown to potentiate ACh-mediated $\alpha 7$ nAChR activation (90).

DYSREGULATION OF AUTONOMIC NERVOUS SYSTEM IN RHEUMATOID ARTHRITIS

As discussed, both the SNS and PNS, and also the HPA axis, influence immune homeostasis. Inflammatory mediators signal to the brain via the circulation or via afferent fibers of the vagus nerve, thereby activating the SNS and/or PNS (Figure 1E). Efferent SNS and vagal nerve fibers then induce local catecholamine and ACh production by neurons or nonneuronal cells. The final effects are difficult to predict, as there are many different signaling molecules and receptors involved depending on the disease phase.

The autonomic dysfunction in RA patients is characterized by an increased overall sympathetic tone and decreased activity of the vagus nerve. This indicates that the normal equilibrium, where the SNS and PNS act oppositely and have contrary effects, is in imbalance and this disturbance might contribute to the induction and/or persistence of the disease (19,91–95). When immune homeostasis is disturbed, both the SNS and HPA axis are activated to restore this. In RA patients, however, inadequately low levels of cortisol were seen in relationship to inflammation and controlled physiological stress (96). These findings led to the assumption that the HPA axis and SNS have been uncoupled in these patients, resulting in a strong proinflammatory situation (30,96). Other changes also take place, for example, density of sympathetic nerve fibers in arthritic patients are decreased (14), while SNS fibers in the spleen redistribute toward the red pulp where the immune cells reside, suggesting there is an altered sympathetic-to-immune system signaling in RA.

Simultaneously, low tone of the vagus nerve means low activity of the cholinergic antiinflammatory pathway, which also can lead to higher cytokine levels,

thereby contributing to this proinflammatory status. Of note, surgical unilateral vagotomy for gastric disease in humans had no effect on the risk of developing RA, but vagotomy at a different anatomical level might have resulted in a different result (97). When considering the parasympathetic-to-immune system signaling, our data show that the cholinergic antiinflammatory pathway contributes to control of disease activity in experimental arthritis, since interruption of this pathway can lead to aggravation of arthritis. Our findings support and extend the pioneering work by KJ Tracey, suggesting that tonic activity of the vagus nerve is essential to maintain immune homeostasis (62). Impairment of this activity could lead to unrestrained cytokine responses and damage to the host.

It is unknown whether the dysregulation of the autonomic nervous system is initiated by an increase in SNS activity (for example, stress) followed by decrease in vagus nerve activity, or if it is the other way around. Regardless, based on experimental results stated above, it seems likely that the autonomic imbalance observed in experimental arthritis and RA patients is, at least partially, responsible for sustaining the inflammatory status. Further insight into this autonomic dysregulation in RA is essential to determine how and to what extent it contributes to the development, persistence and exacerbation of arthritis.

POTENTIAL CLINICAL IMPLICATIONS FOR MODULATION OF THE NERVE SYSTEM IN INFLAMMATORY DISEASES

The immunoregulatory potential of the SNS has led to the proposition of new antiinflammatory therapeutic approaches for RA. Based on animal models discussed above, it can be envisaged that intervening with SNS activity can influence inflammation in RA. Until now, no clinical testing of AR agents as a treatment option for RA patients has been performed. Since data targeting AR receptors obtained from animal models are somewhat variable, it is difficult to draw definitive conclusions as to which treat-

ment would be beneficial in arthritis patients.

The PNS can exert its antiinflammatory effects via the vagal cholinergic anti-inflammatory pathway in which the neurotransmitter acetylcholine plays an important role. The discovery of the cholinergic antiinflammatory pathway has paved the way for new therapeutic strategies for inflammation-mediated diseases like RA. The two major treatment options are pharmacological intervention or electric stimulation of the vagus nerve (VNS). The cholinergic anti-inflammatory pathway can be stimulated by pharmacologic activation of the nAChRs. The observation that specific nAChR α 7 agonists diminish disease in several animal models of inflammation, including animal models for arthritis (78,79) and experimental colitis (98), suggests that therapeutic agents that can modify cholinergic signaling might be beneficial in humans. Nicotine, a potent nAChR α 7 agonist, has been used extensively to examine the cholinergic anti-inflammatory pathway in animal models. However, the therapeutic value of nicotine is limited, because of its lack of specificity and toxicity. To avoid side effects caused by nicotine, more specific nAChR agonists have been designed. The most extensively characterized nAChR specific agonist is GTS-21, a nAChR α 7 agonist that also affects 42 nAChR. GTS-21 is well tolerated in healthy volunteers as well as in patients with schizophrenia and Alzheimer's disease (99). In addition, there is evidence indicating that centrally acting cholinergic drugs used in treatment of Alzheimer's patients can modulate peripheral immune responses, which make this group of drugs interesting to explore in inflammatory disorders (100).

A novel antiinflammatory strategy also could be developed by means of optimal VNS generated by a special device. VNS has recently been shown to inhibit development of CIA in rats using a constant voltage stimulus (5 V, 2 ms, 1 Hz) starting at day 10 after the second immunization (101). Vagus nerve stimulation al-

ready is used in patients with drug-resistant epilepsy and depression. The left vagus nerve is stimulated via an implantable electrode. Vagus nerve stimulation had beneficial effects in both disorders without major side effects (99). A recent study investigating in more detail the effect of VNS on the immune system in 11 patients with refractory epilepsy demonstrated that VNS causes a rebalancing of the immune system compared with a control group (102). The effects of VNS on pro- and antiinflammatory cytokines in peripheral blood observed in this study in combination with the results found in the rat model of arthritis suggest that VNS could be a promising strategy in the treatment of RA patients.

Overall, data obtained from a large number of *in vitro* and *in vivo* studies imply that therapeutic agents targeting the PNS via the cholinergic antiinflammatory pathway or targeting the SNS via AR receptors can be an important future treatment option in a variety of conditions. However, further preclinical and clinical studies are needed to further explore the potential and safety of these approaches in patients with inflammatory disorders.

ACKNOWLEDGMENTS

Supported by Dutch Arthritis Association grant NR 09-1-307. In addition, the authors would like to thank Beatrijs M Lodde for editorial assistance.

DISCLOSURE

PP Tak received research support from SetPoint and Critical Therapeutics.

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