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Word count : 2247

Keywords : vagus nerve, vagus nerve stimulation, pain, inflammation, arthritis, rheumatoid arthritis

Abstract (211 words)

The vagus nerve is the main nerve of the parasympathetic autonomic nervous system. Beyond its vegetative functions, the vagus nerve possesses anti-inflammatory and analgesic properties. Initially developed in the treatment of refractory epilepsy, vagus nerve stimulation (VNS) is currently being evaluated in several musculoskeletal diseases. VNS can be invasive by placing an electrode around the cervical vagus nerve and connected to a generator implanted subcutaneously or non-invasive stimulating the cervical vagus nerve branch percutaneously (auricular or cervical). In rheumatoid arthritis (RA) patients, VNS has been shown to dampen the inflammatory response of circulatory peripheral cells. Several open-labeled small pilot studies have demonstrated that VNS, either invasive or transcutaneous, is associated with a significant decrease of RA disease activity. As well, other studies have shown that VNS could limit fatigue in Sjogren's syndrome and systemic lupus, or decrease pain in fibromyalgia as well as in erosive hand osteoarthritis. However, some questions remain, such as the settings of stimulation, the duration of treatment, or the optimal stimulation route. Finally, randomized controlled trials *versus* sham stimulation with large samples of patients are mandatory to definitively conclude about the efficacy of VNS.

1. Introduction

Vagus nerve belongs to the autonomic parasympathetic nervous system regulating vegetative functions but can also modulate inflammation. Activation of the vagus nerve reduces systemic inflammation by decreasing the splenic and peripheral response to inflammatory stress [1,2]. This effect is due to a complex loop, called the cholinergic anti-inflammatory pathway (CAP), which has required more than 20 years of research but still raises some questions. The CAP highlights the complex relationship between the nervous system and the immune system[3]. Moreover, beyond its anti-inflammatory properties, vagus nerve, through cerebral afferences connected with central pain centers, displays analgesic properties too. This is why vagus nerve stimulation (VNS), which was used in refractory epileptic patients for several years, has become a new therapeutic avenue in several inflammatory or painful disorders such as inflammatory bowel diseases or musculoskeletal diseases [4,5].

2. Cholinergic anti-inflammatory pathway

The vagus nerve is the main nerve of the parasympathetic autonomic nervous system. It is a sensory, motor and vegetative nerve. All these functions are possible thanks to the secretion of acetylcholine (Ach) and its fixation on the Ach receptors. Among them, nicotinic receptors, discovered in the 1970s by Changeux et al., were shown much more recently to be involved in the control of systemic inflammation [6]. Nicotinic receptors are ligand-gated ion channels receptors composed of five identical or different subunits between α1-10 and β1-4. To date , the main nicotinic receptor involved in the anti-inflammatory effect of the CAP is the homopentameric alpha 7 nicotinic Ach receptor (α7nAchR) [7].

Tracey et al. discovered in the 2000s that, during septic shock in rats, VNS, not only dampened the systemic and hepatic production of tumor necrosis factor (TNF), but also decreased the severity of the septic shock [1]. Several years were then necessary to fully understand the

precise mechanism of such a phenomenon. In fact, neuronal and non-neuronal Ach production are both required. Briefly, stimulation of the efferences of the vagus nerve (i.e., from the brainstem to the peripheral tissues) will cause the decrease of pro-inflammatory cytokines release by splenic macrophages through the activation of the α7nAChR by Ach. Since the spleen is not directly innervated by the vagus nerve, this anti-inflammatory effect involves several intermediates (splenic nerve, splenic T-cells) (**Figure 1**) [7–10].

Afferences of the vagus nerve (i.e., from peripheral tissues to the brainstem) also participate to the control of inflammation. Vagus nerve afferent fibers are sensitive to peripheral cytokines (TNF, IL-1β and IL-6). Watkins et al. found that peripheral neurons can detect the presence of inflammation in damaged tissues [11,12] and activate the vagus nerve afference. Afferences connect and activate the hypothalamo-hypophyseal-adrenal (or hypothalamo-pituitary-adrenal) axis that participates to the CAP by the release of cortisol by the adrenal gland (**Figure 1**). Finally, afferences of vagus nerve also project in several other cerebral centers that may also participate in the control of pain.

Some unknowns and some debates still remain, especially about the involvement of the splenic nerve. However, altogether, these data support to test VNS as a therapy in many inflammatory and chronic pain diseases.

3. Vagus nerve stimulation modalities

VNS can be done invasively by cervical implantable stimulating devices targeting the cervical portion vagus nerve. This has been done for decades in patients with refractory epilepsy and more recently in patients with depression [13]. Recent non-invasive methods were developed of transcutaneous stimulation at two different anatomical locations: at the cymba conchae of the ear where is located one sensitive afferent branch of the vagus nerve or at the neck containing the cervical vagus nerve (**Figure 2**). Both methods were shown to stimulate the

central projection area of vagus nerve using functional MRI and are recognized as effective VNS [14,15].

4. Vagus nerve stimulation in rheumatoid arthritis

In musculoskeletal diseases, VNS has been first developed in rheumatoid arthritis (RA) (**Table 1**). Pre-clinical data confirmed that vagotomy exacerbated arthritis in mice [16]. This effect was also supposed to be related to the α7nAChR since the genetic deletion of the α7nAChR resulted in the exacerbation of arthritis [17]. Conversely, VNS of arthritis-induced rats counteracted inflammation and arthritis [18].

Several studies demonstrate that RA patients had lower vagal tone compared to controls [19–21] and that a lower vagal tone at baseline is associated with a poor response to anti-TNF in RA and psoriatic arthritis [22]. The vagal or parasympathetic tone is observed on a long ECG by quantifying the heart rate variability or more easily by the heart rate at rest, in absence of confounding factors such as tobacco or cholinergic drugs.

Tracey et al. evaluated the whole-blood response to endotoxemia before and after invasive vagal stimulation in seven epileptic patients without any rheumatic diseases. After VNS, the endotoxin-induced whole blood release of TNF, IL-6 and IL-1β was significantly decreased [2]. This was the starting point for the first human trial of VNS in RA (NCT01552941). The first pilot study of invasive VNS has included 17 patients with active and established RA non responsive to methotrexate. Most of the patients (83%) received at least one biologic Disease Modifying Anti-Rheumatic Drugs (DMARDs) before inclusion. After surgical procedure and a 14-day postoperative recovery period, the VNS was performed on day 1 for 60 seconds to determine the intensity of stimulation. At day 7, patients were stimulated every day until day 42. Invasive VNS decreased significantly whole blood release of TNF induced by lipopolysaccharide (LPS) in RA patients. To eliminate the regression effect to the mean, the

VNS was then stopped at day 42 for a period of 14 days and then reactivated from day 56 to day 84. LPS-induced TNF production increased significantly after interrupting the stimulation and decreased again when the VNS was resumed. In addition to the biological effects, DAS28-CRP at day 42 decreased significantly compared to baseline. Concerning the safety, there were no death or infection but 50% had adverse events related to the procedure such as dysphonia, hypoesthesia or dyspnea [2]. After 24 months of follow-up, 5 patients did not respond to VNS and 4 responded although not achieved remission. Therefore, biological DMARDs were initiated in association with VNS in these 9 patients while 8 patients kept VNS alone. There was no safety signal during a 24-month period of VNS (NCT01552538) [23]. To limit adverse events, a new and miniaturized device of invasive cervical VNS has been recently tested in RA patients: 6 received 1 stimulation by day (qd), 4 patients received 1 stimulation 4 times by day (qid) and finally 4 patients had a sham stimulation (devices implanted but not activated) (NCT03437473). Two transient but noticeable side effects have been reported: one Horner's syndrome and one vocal cord paralysis. After 12 weeks of stimulation, while DAS28-CRP did not change in the sham group and in the qid stimulation, it decreased in the group stimulated qd. However, the number of patients per group did not allow any statistical analysis [24].

A large randomized double blind controlled study evaluating invasive VNS in moderate and severe RA should start soon (NCT04539964). Invasive VNS raises safety issues related to the surgical procedure and is non-removable which can be problematic in case of MRI for example [25]. This is why there is now a great interest in non-invasive stimulation in RA.

Three recent pilot open-labeled studies evaluated the transcutaneous VNS (tVNS) in RA, especially at the auricular site. First, Tracey et al. confirmed that a vibrotractile device tVNS at the cymba conchae reduced significantly the LPS-induced TNF, IL-6 and IL-1β releases in healthy controls, with a similar biological effect than invasive VNS (NCT00859859). They also

demonstrated that tVNS twice a day (bid) during 2 days significantly diminished the DAS-28 CRP in 9 patients with RA at day 2 and 7 [26]. Auricular tVNS was also evaluated on a longer term (i.e., 3 months) and decreased also RA activity. From the 30 RA patients treated with tVNS for 3 months, 11 patients reached low disease activity and 7 achieved remission. As well, ACR20/50/70 response rates were 53%, 33%, and 17% respectively at 3 months. Tolerance was good during the follow-up period [27]. Finally, the cervical tVNS performed 3 times by day (tid) for 4 days decreased significantly the DAS-28 CRP in RA patients with high disease activity (4.1 to 3.8, $p = 0.02$; *n=16*) with a decrease of objective data such as CRP ($p>0.01$) and swollen joints ($p=0.05$). No effect was observed in RA patients with low disease activity (*n=20*) [28].

The proof of concept of the interest of VNS in RA, whether invasive or transcutaneous and at short and mid-term, has now been demonstrated in several pilot studies opening the way to randomized controlled studies *versus* sham stimulation. Sham vagus nerve stimulation might be challenging and must be adequately managed before setting up the study (**Table 2**).

Author	Type of VNS	Number of Patients	Follow-up	Results
Koopman 2016 [2] and 2018 [23]	Invasive between day 7 and day 42	17	84 days	• Significant decrease of LPS-induced TNF production • Significant decrease of DAS-28 CRP (6.05 ± 0.18 at baseline *versus* 4.16 ± 0.39 on day 42, P < 0.001)
			24 months	• Biologics DMARDs initiated in 9/17 patients • Mean DAS-28 CRP of the 8 patients with VNS alone 3.76±1.77
Addorisio 2019 [26]	Vibrotactile auricular tVNS for 2 days	9	7	• Significant decrease of DAS-28 CRP (4.19 ± 0.33 at baseline versus 3.12 ± 0.25 at day 2, $p<0.05$ and 2.79 ± 0.21 at day 7, $p<0.01$) • Significant reduction of CRP levels at day 2, of VAS global health at day 2 and day 7.
Marsal 2020 [27]	Auricular tVNS for 12 weeks	30	12 weeks	• Significant decrease of the DAS-28 CRP (-1.4; $p< 0.001$) with decrease of all DAS-28 CRP component except for CRP • 11/30 achieved low disease activity and 7 remission • Significant change in HAQ-DI (-0.47; $p< 0.05$)
Drewes 2020 [28]	Cervical tVNS for 4 days	36	4 days	• In the high disease activity RA group, significant decrease of DAS-28 CRP (4.1 to 3.8, $p = 0.02$), of CRP and IFNγ • No effect in the low disease activity RA group
Genovese 2020 [24]	Cervical invasive VNS for 12 weeks	14	12 weeks	• Decreased of DAS-28 CRP in 4/6 patients stimulated once a day but no effect of VNS stimulation four times by day and of sham group • One transient Horner's syndrome and vocal cord paralysis

Table 1 : Results of human trials evaluating the vagus nerve stimulation in Rheumatoid Arthritis. *DMARDs : Disease Modifying Anti-Rheumatic Drug; TNF : Tumor Necrosis Factor,*

RA : Rheumatoid arthritis; HAQ-DI : Health assessment questionnaire disability index; IFNγ : Interferon-gamma; DAS-28 CRP : Disease Activity Score 28 C Reactive Protein; VAS : Visual Analog Scale

	Sham procedure	Advantage (+) or disadvantage (-) of each procedure
Invasive vagus nerve stimulation	Sham surgery without device implantation	(+) less invasive than with implanted device
		(-) could raise ethics and blinding issues
	Implanted device but without any stimulation	(+) accurate blinding, allow cross over studies
		(-) risk of the procedure
Non invasive vagus nerve stimulation	Stimulation at another location not innervated by the vagus nerve (the earlobe for example)	(+) paresthesia similar between active and sham group
		(-) risk of blind lifting by the patient and the investigator, possible vagal innervation in near areas of the cymba concha
	Same device but without electric stimulation	(+) appropriate double blinding (patient and investigator) of the treatment group during the study
		(-) risk of blind lifting because of paresthesia experience different between both groups (do not use in cross over studies)
	Same active device but with other settings (modified frequency, very short stimulation)	(+) better blinding procedure
		(-) cannot eliminate effective vagus nerve stimulation

Table 2 : Possibilities of sham procedure for invasive and non-invasive vagus nerve stimulations with advantage and disadvantage of each procedure.

5. Vagus nerve stimulation in other autoimmune musculoskeletal disorders

The efficacy of VNS has been evaluated in other autoimmune diseases in few small-sized studies. First, Tran et al. explored the effect of cervical tVNS on immune response and on symptoms in 15 patients with primary Sjögren's syndrome (pSS). After a bid stimulation for 26 days, cervical tVNS significantly reduced the whole blood LPS-induced production of IL-6, IL-1β and TNF and improved fatigue. No significant effect was shown on hospital anxiety and depression (HAD) or quality of life scales [29]. As well, auricular tVNS efficacy on musculoskeletal pain and fatigue has been evaluated in patients with systemic lupus erythematosus at very short term (4 days) (NCT02822989). Here, there was a control group with sham stimulation (*n=12* in treatment group and *n=6* in sham group). Sham procedure was the same electrode applied on a zone innervated by the vagus nerve but without stimulation. After four consecutive stimulations, subjects receiving tVNS achieved a significantly greater

reduction in pain compared with the sham group (median [interquartile] -5.00 [-5.8 to -3.1] *versus* 0.10 [-10.0 to 1.0], p=0.049). Fatigue, patient global assessment, number of tender and swollen joints as well as plasma concentration of the neuropeptide substance P were also significantly ameliorated in the tVNS group compared to the sham group [30]. These promising results need to be confirmed in larger randomized studies.

6. Vagus nerve stimulation in spondyloarthritis

There is no available pilot open-labeled study of VNS in spondyloarthritis. However, based on the previous literature on the effect on VNS on other inflammatory rheumatic diseases, a large French study of tVNS in spondyloarthritis has been registered recently in clinical.gov (NCT04286373). The objective of this cross-over randomized and placebo controlled study is to evaluate the efficacy of auricular tVNS on SpA activity (ASAS 20 response) in 102 participants.

7. Vagus nerve stimulation in fibromyalgia

Through its afferent fibers going to the brain structures, VNS delivers also analgesic effects and has been evaluated in migraine [31]. Thus, it made sense to evaluate it in fibromyalgia. A trial with invasive VNS has been performed in 14 patients with fibromyalgia (NCT00294281). As for epilepsy or RA studies, adverse events in relation with the procedure occurred (eg, dyspepsia, neuropathic pain around the stimulator). At 3 month, five participants fulfilled the efficacy criteria (significant decrease of pain). The therapeutic effect seemed to increase over time because additional participants attained efficacy criteria at 11 months [32]. However, since 2011, no randomized studies have been published or are planned according to the clinical.gov website.

8. Vagus nerve stimulation in osteoarthritis

Considering the non-superiority over placebo of many drugs in osteoarthritis (OA), VNS could be a new non-pharmacological strategy in this disease. Pre-clinical study confirmed that the absence of α7nAChR was associated with more severe OA lesions as in RA [33] . Moreover, cholinergic nerves are found in subchondral bone of knee OA patients [34]. We recently conducted an open label pilot study of auricular tVNS in erosive hand OA (EHOA), a classical model of inflammatory OA with high pain intensity (NCT03919279). Eighteen patients with symptomatic EHOA and with at least one interphalangeal swollen joint were included. Auricular tVNS (1 hour/day) significantly reduced VAS hand pain, with a median [interquartile] decrease of 23.5mm [7.7 to 37.2], p=0.001 and the Functional Index of Hand OA score (FIHOA) of 2 points [0.75 to 5.2] decrease, p=0.01 after 4 weeks of use. Tolerance was good apart from some local side effects such as tingling [35]. This study allowed us to set up a large French multicentric randomized controlled trial (ESTIVAL, NCT04520516) *versus* sham stimulation which will be started in 2021.

9. Other possibilities of cholinergic anti-inflammatory pathway activation

Beyond VNS stimulation, activation of the CAP could also be achieved by other methods. A recent study has shown that the CAP could be activated by the ultrasound (US) stimulation of the spleen using daily US low frequency (1Mhz, at 350 kPa for 2 min) reducing arthritis in mice [36]. As well, systemic adjunction of α7nAchR agonists could be a therapeutic option although this has not been studied in inflammatory diseases yet. However, α7nAchR is composed of five α7 subunit encoded by Chrna7. Chrna7 have a specific human dominant negative duplicate called CHRFAM7A that encoded for a truncated and less-effective subunit [37]. Evaluation of the expression of this duplicate in patients could modulate the response to the VNS or to the α7nAchR agonists [38,39].

10. Conclusion

In conclusion, beyond its autonomic functions, VNS displays anti-inflammatory and analgesic properties through the CAP. Therefore, VNS represents a potential novel therapeutic strategy in many musculoskeletal diseases with inflammation and/or pain (**Table 3**). The use of a non-invasive device is easier, less expensive and much better tolerated. While a significant number of pilot studies have established proof of concept of VNS efficacy in chronic musculoskeletal diseases, there is now a strong need for randomized controlled trials. Only these trials will definitively establish whether VNS is a therapeutic option and in which indications.

Table 3 : Research Agenda

Research Agenda
Randomized controlled trials evaluating VNS (invasive or non-invasive) in rheumatoid arthritis, Sjogren syndrome and systemic lupus
To better decipher the action of the vagus nerve on pain mechanisms
To better determine the dose and the frequency of VNS stimulation
To elucidate the best sham procedure of non-invasive VNS (electrode at the same place but with no stimulation or at other locations with stimulation)
To determine the influence of the expression of the dominant alpha 7 nicotinic receptor on VNS response
To determine the tolerance and efficacy of systemic adjunction alpha 7 nicotinic agonists in inflammatory diseases

Disclosure of interest : The authors declare that they have no competing interest.

Figure captions :

Figure 1 : Cholinergic anti-inflammatory pathway.
Cytokines release activates afferent fibers of the vagus nerve through paraganglia structure. Afferent neuronal signals from the vagus nerve are relayed to vagus nerve projection centers (nucleus of the solitary tract (NTS) and others). It activates efferent vagus nerve fibers that releases acetylcholine (Ach) in the coeliac ganglia and make a synapse with the sympathetic splenic nerve. The splenic nerve release norepinephrine (NE) that binds to Beta-2adrenergic receptors (β2-AR) of the splenic T-cells. NE induces non neuronal production of Ach by T-cells. Ach binds to the alpha 7 nicotinic Ach receptor (α7 nAchR) expressed by macrophages which leads to a decrease of cytokine production by splenic macrophages. Vagus nerve afferents also stimulate the hypothalamic-hypohyso-adrenal (HPA) axis and cause the release of cortisol by the adrenal gland which also participates to counteract inflammation.

Figure 2: Different possibilities of vagus nerve stimulation. *A: implantable cervical device with electrode applied on the vagus nerve and generator in the chest, from Koopman FA et al, Best practice & Research Clinical Rheumatology, 2014. B: Auricular transcutaneous VNS using an electrode connected to a TENS device (Schwa Medico). C: Cervical device for transcutaneous VNS (Copyright electroCore, Inc. All rights reserved. Used with the permission of electroCore, Inc.)*

References

[1] Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 2000;405:458–62. https://doi.org/10.1038/35013070.

[2] Koopman FA, Chavan SS, Miljko S, Grazio S, Sokolovic S, Schuurman PR, et al. Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis. Proc Natl Acad Sci USA 2016;113:8284–9. https://doi.org/10.1073/pnas.1605635113.

[3] Huston JM, Tracey KJ. The pulse of inflammation: heart rate variability, the cholinergic anti-inflammatory pathway and implications for therapy. J Intern Med 2011;269:45–53. https://doi.org/10.1111/j.1365-2796.2010.02321.x.

[4] Bonaz B, Sinniger V, Hoffmann D, Clarençon D, Mathieu N, Dantzer C, et al. Chronic vagus nerve stimulation in Crohn's disease: a 6-month follow-up pilot study. Neurogastroenterol Motil 2016;28:948–53. https://doi.org/10.1111/nmo.12792.

[5] Sinniger V, Pellissier S, Fauvelle F, Trocmé C, Hoffmann D, Vercueil L, et al. A 12-month pilot study outcomes of vagus nerve stimulation in Crohn's disease. Neurogastroenterol Motil 2020;32:e13911. https://doi.org/10.1111/nmo.13911.

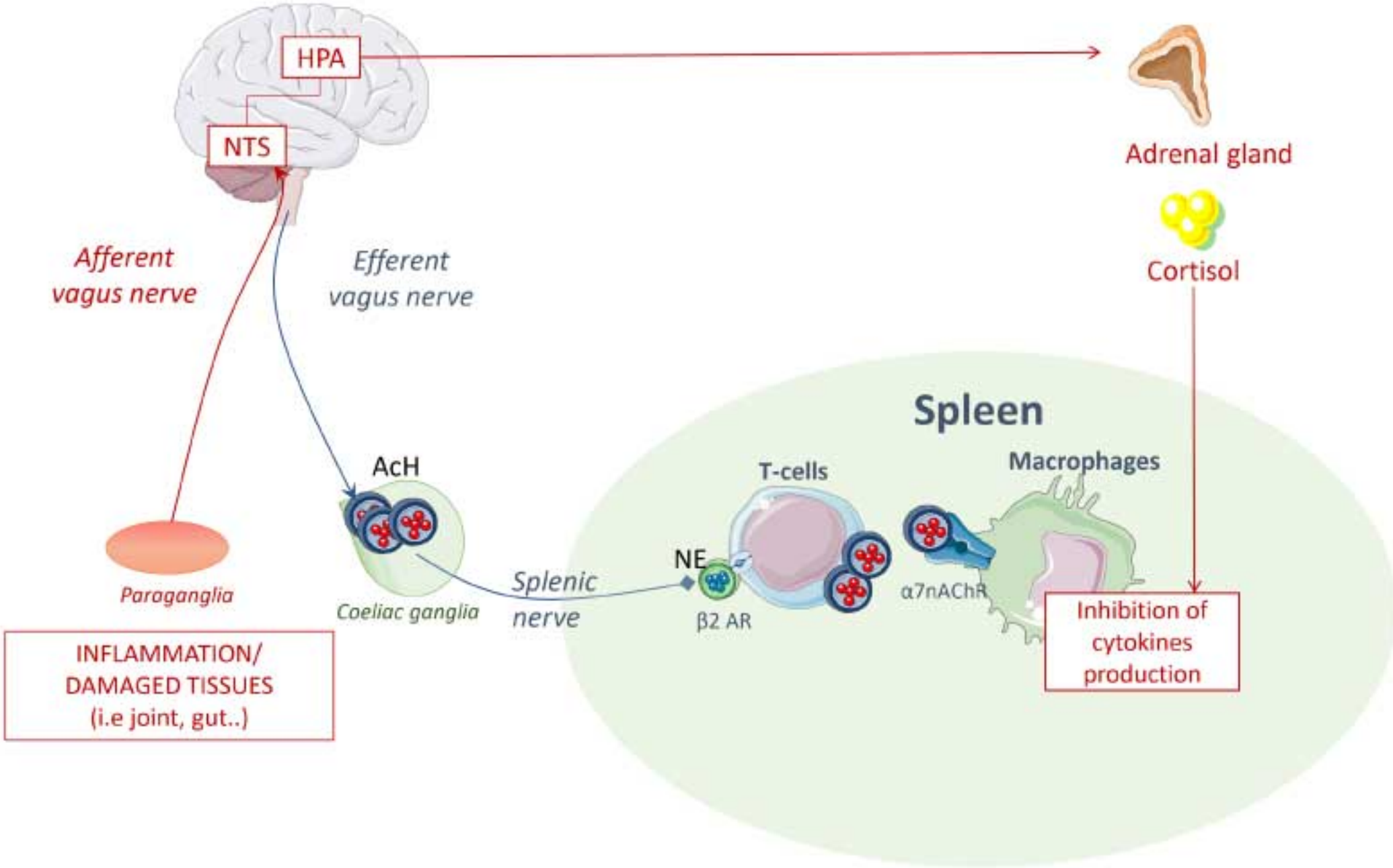
[6] Changeux J-P. Golden Anniversary of the Nicotinic Receptor. Neuron 2020;107:14–6. https://doi.org/10.1016/j.neuron.2020.05.026.

[7] Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 2003;421:384–8. https://doi.org/10.1038/nature01339.

[8] Berthoud HR, Powley TL. Characterization of vagal innervation to the rat celiac, suprarenal and mesenteric ganglia. J Auton Nerv Syst 1993;42:153–69.
[9] Rosas-Ballina M, Ochani M, Parrish WR, Ochani K, Harris YT, Huston JM, et al. Splenic nerve is required for cholinergic antiinflammatory pathway control of TNF in endotoxemia. Proc Natl Acad Sci USA 2008;105:11008–13. https://doi.org/10.1073/pnas.0803237105.
[10] Rosas-Ballina M, Olofsson PS, Ochani M, Valdés-Ferrer SI, Levine YA, Reardon C, et al. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. Science 2011;334:98–101. https://doi.org/10.1126/science.1209985.
[11] Watkins LR, Goehler LE, Relton JK, Tartaglia N, Silbert L, Martin D, et al. Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. Neurosci Lett 1995;183:27–31. https://doi.org/10.1016/0304-3940(94)11105-r.
[12] Hansen MK, O'Connor KA, Goehler LE, Watkins LR, Maier SF. The contribution of the vagus nerve in interleukin-1beta-induced fever is dependent on dose. Am J Physiol Regul Integr Comp Physiol 2001;280:R929-934. https://doi.org/10.1152/ajpregu.2001.280.4.R929.
[13] Milby AH, Halpern CH, Baltuch GH. Vagus nerve stimulation for epilepsy and depression. Neurotherapeutics 2008;5:75–85. https://doi.org/10.1016/j.nurt.2007.10.071.
[14] Frangos E, Ellrich J, Komisaruk BR. Non-invasive Access to the Vagus Nerve Central Projections via Electrical Stimulation of the External Ear: fMRI Evidence in Humans. Brain Stimul 2015;8:624–36. https://doi.org/10.1016/j.brs.2014.11.018.
[15] Frangos E, Komisaruk BR. Access to Vagal Projections via Cutaneous Electrical Stimulation of the Neck: fMRI Evidence in Healthy Humans. Brain Stimul 2017;10:19–27. https://doi.org/10.1016/j.brs.2016.10.008.
[16] van Maanen MA, Lebre MC, van der Poll T, LaRosa GJ, Elbaum D, Vervoordeldonk MJ, et al. Stimulation of nicotinic acetylcholine receptors attenuates collagen-induced arthritis in mice. Arthritis Rheum 2009;60:114–22. https://doi.org/10.1002/art.24177.
[17] van Maanen M, Stoof SP, Larosa G, Vervoordeldonk M, Tak P. Role of the cholinergic nervous system in rheumatoid arthritis: aggravation of arthritis in nicotinic acetylcholine receptor α7 subunit gene knockout mice. Annals of the Rheumatic Diseases 2010;69:1717–23. https://doi.org/10.1136/ard.2009.118554.
[18] Levine YA, Koopman FA, Faltys M, Caravaca A, Bendele A, Zitnik R, et al. Neurostimulation of the cholinergic anti-inflammatory pathway ameliorates disease in rat collagen-induced arthritis. PLoS ONE 2014;9:e104530. https://doi.org/10.1371/journal.pone.0104530.
[19] Provan SA, Olstad DS, Solberg EE, Smedslund G, Dagfinrud H. Evidence of reduced parasympathetic autonomic regulation in inflammatory joint disease: A meta-analyses study. Semin Arthritis Rheum 2018;48:134–40. https://doi.org/10.1016/j.semarthrit.2017.11.010.
[20] Koopman FA, Tang MW, Vermeij J, de Hair MJ, Choi IY, Vervoordeldonk MJ, et al. Autonomic Dysfunction Precedes Development of Rheumatoid Arthritis: A Prospective Cohort Study. EBioMedicine 2016;6:231–7. https://doi.org/10.1016/j.ebiom.2016.02.029.
[21] Bruchfeld A, Goldstein RS, Chavan S, Patel NB, Rosas-Ballina M, Kohn N, et al. Whole blood cytokine attenuation by cholinergic agonists ex vivo and relationship to vagus nerve activity in rheumatoid arthritis. J Intern Med 2010;268:94–101. https://doi.org/10.1111/j.1365-2796.2010.02226.x.
[22] Holman AJ, Ng E. Heart rate variability predicts anti-tumor necrosis factor therapy response for inflammatory arthritis. Auton Neurosci 2008;143:58–67. https://doi.org/10.1016/j.autneu.2008.05.005.
[23] Koopman FA, Musters A, Backer M, Gerlag D, Miljko S, Grazio S, et al. Vagus Nerve Stimulation in Patients with Rheumatoid Arthritis: 24 Month Safety and Efficacy.

Arthritis Rheumatol 2018;70.
[24] Genovese MC, Gaylis NB, Sikes D, Kivitz A, Horowitz DL, Peterfy C, et al. Safety and efficacy of neurostimulation with a miniaturised vagus nerve stimulation device in patients with multidrug-refractory rheumatoid arthritis: a two-stage multicentre, randomised pilot study. The Lancet Rheumatology 2020;2:e527–38. https://doi.org/10.1016/S2665-9913(20)30172-7.
[25] Koopman FA, Schuurman PR, Vervoordeldonk MJ, Tak PP. Vagus nerve stimulation: a new bioelectronics approach to treat rheumatoid arthritis? Best Pract Res Clin Rheumatol 2014;28:625–35. https://doi.org/10.1016/j.berh.2014.10.015.
[26] Addorisio ME, Imperato GH, de Vos AF, Forti S, Goldstein RS, Pavlov VA, et al. Investigational treatment of rheumatoid arthritis with a vibrotactile device applied to the external ear. Bioelectron Med 2019;5:4. https://doi.org/10.1186/s42234-019-0020-4.
[27] Marsal S, Corominas H, De Agustin De Oro J, Perez Garcia C, Lopez Lasanta M, Borrell H, et al. Non-invasive Vagus Nerve Stimulation Improves Signs and Symptoms of Rheumatoid Arthritis: Results of a Pilot Study [abstract]. Arthritis Rheumatol 2020;72:Suppl 10.
[28] Drewes AM, Brock C, Rasmussen SE, Møller HJ, Brock B, Deleuran BW, et al. Short-term transcutaneous non-invasive vagus nerve stimulation may reduce disease activity and pro-inflammatory cytokines in rheumatoid arthritis: results of a pilot study. Scand J Rheumatol 2020:1–8. https://doi.org/10.1080/03009742.2020.1764617.
[29] Tarn J, Legg S, Mitchell S, Simon B, Ng W-F. The Effects of Noninvasive Vagus Nerve Stimulation on Fatigue and Immune Responses in Patients With Primary Sjögren's Syndrome. Neuromodulation 2019;22:580–5. https://doi.org/10.1111/ner.12879.
[30] Aranow C, Atish-Fregoso Y, Lesser M, Mackay M, Anderson E, Chavan S, et al. Transcutaneous auricular vagus nerve stimulation reduces pain and fatigue in patients with systemic lupus erythematosus: a randomised, double-blind, sham-controlled pilot trial. Ann Rheum Dis 2020. https://doi.org/10.1136/annrheumdis-2020-217872.
[31] Yuan H, Silberstein SD. Vagus Nerve Stimulation and Headache. Headache 2017;57 Suppl 1:29–33. https://doi.org/10.1111/head.12721.
[32] Lange G, Janal MN, Maniker A, Fitzgibbons J, Fobler M, Cook D, et al. Safety and efficacy of vagus nerve stimulation in fibromyalgia: a phase I/II proof of concept trial. Pain Med 2011;12:1406–13. https://doi.org/10.1111/j.1526-4637.2011.01203.x.
[33] Courties A, Do A, Leite S, Legris M, Sudre L, Pigenet A, et al. The Role of the Non-neuronal Cholinergic System in Inflammation and Degradation Processes in Osteoarthritis. Arthritis Rheumatol 2020. https://doi.org/10.1002/art.41429.
[34] Courties A, Belle M, Senay S, Cambon-Binder A, Sautet A, Chédotal A, et al. Clearing method for 3-dimensional immunofluorescence of osteoarthritic subchondral human bone reveals peripheral cholinergic nerves. Sci Rep 2020;10:8852. https://doi.org/10.1038/s41598-020-65873-6.
[35] Courties A, Deprouw C, Maheu E, Gibert E, Gottenberg J-E, Champey J, et al. Effect of Transcutaneous Vagal Nerve Stimulation in Erosive Hand Osteoarthritis: Results from an Open-label Non-randomized Pilot Trial [abstract]. Arthritis Rheumatol 2020;72:Suppl 10.
[36] Zachs DP, Offutt SJ, Graham RS, Kim Y, Mueller J, Auger JL, et al. Noninvasive ultrasound stimulation of the spleen to treat inflammatory arthritis. Nat Commun 2019;10:951. https://doi.org/10.1038/s41467-019-08721-0.
[37] Costantini TW, Dang X, Coimbra R, Eliceiri BP, Baird A. CHRFAM7A, a human-specific and partially duplicated α7-nicotinic acetylcholine receptor gene with the potential to specify a human-specific inflammatory response to injury. J Leukoc Biol 2015;97:247–57. https://doi.org/10.1189/jlb.4RU0814-381R.
[38] Szigeti K, Ihnatovych I, Birkaya B, Chen Z, Ouf A, Indurthi DC, et al. CHRFAM7A:

A human specific fusion gene, accounts for the translational gap for cholinergic strategies in Alzheimer's disease. EBioMedicine 2020;59:102892. https://doi.org/10.1016/j.ebiom.2020.102892.
[39] Courties A, Boussier J, Hadjadj J, Yatim N, Barnabei L, Péré H, et al. Regulation of the acetylcholine/α7nAChR anti-inflammatory pathway in COVID-19 patients. Submitted n.d.


HPA
NTS
Adrenal gland
Cortisol
Afferent vagus nerve
Efferent vagus nerve
Spleen
AcH
T-cells
Macrophages
NE
β2 AR
α7nAChR
Splenic nerve
Paraganglia
Coeliac ganglia
Inhibition of cytokines production
INFLAMMATION/ DAMAGED TISSUES (i.e joint, gut..)


A
B
C