

The role of the cholinergic anti-inflammatory pathway in autoimmune rheumatic diseases

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Abstract

The cholinergic anti-inflammatory pathway (CAP) is a classic neuroimmune pathway, consisting of the vagus nerve, acetylcholine (ACh)—the pivotal neurotransmitter of the vagus nerve—and its receptors. This pathway can activate and regulate the activities of immune cells, inhibit cell proliferation and differentiation, as well as suppress cytokine release, thereby playing an anti-inflammatory role, and widely involved in the occurrence and development of various diseases; recent studies have demonstrated that the CAP may be a new target for the treatment of autoimmune rheumatic diseases. In this review, we will summarize the latest progress with the view of figuring out the role of the cholinergic pathway and how it interacts with inflammatory reactions in several autoimmune rheumatic diseases, and many advances are results from a wide range of experiments performed in vitro and in vivo.

1 | INTRODUCTION

1.1 | The cholinergic anti-inflammatory pathway

The cholinergic anti-inflammatory pathway (CAP) is cross-talk between the autonomic nervous system and the immune system, mainly composed of the parasympathetic nerves, in which the vagus nerve plays the most important role, acetylcholine (ACh) and its receptors.¹ This pathway can activate and regulate the activities of immune cells, thereby playing an anti-inflammatory role, and widely involved in the occurrence and development of various diseases. In recent years, the effects of alleviating the inflammatory reactions by the CAP on several diseases, such as haemorrhagic shock, peritonitis, pancreatitis and acute kidney injury, have been deeply studied and demonstrated.^{1,2}

As the main neurotransmitter of the vagus nerve and the pivotal component of CAP, ACh can combine with the nicotinic acetylcholine receptor (nAChR) family to exert anti-inflammatory effects. Wang and his colleagues³ have elucidated that ACh can inhibit HMGB1—a protein highly expressed in extra cellular and facilitates inflammation—release

and improve survival in experimental sepsis via nAChRs. And non-neuronal ACh in lymphocytes can also repress the inflammatory process and T cell proliferation.⁴

nAChRs consist of different muscle-type and neuronal receptors ($\alpha 1$ to $\alpha 10$, $\beta 1$ to $\beta 4$, γ , δ and ϵ),^{1,5} and previous studies of Wang et al,^{3,6} have demonstrated that the nAChR $\alpha 7$ subunit ($\alpha 7$ nAChR), the activation of which is cardinal in CAP, is an essential regulator of the various inflammation responses. $\alpha 7$ nAChR mainly expressed on the membrane of immune cells, including dendritic cells, T and B lymphocytes, monocytes and macrophages.^{5,7,8} It can be activated by vagus nerve stimulants and cholinergic agonists to suppress the release of inflammatory cytokines and attenuate inflammatory reactions.⁹ In models of collagen-induced arthritis and adjuvant-induced arthritis, electrical stimulation of the vagus nerve slowed down the progression of the diseases, while vagotomy surgery exacerbated symptoms and hyperalgesia by enhancing the neutrophil migration.¹⁰ Furthermore, in various animal models of inflammation, the $\alpha 7$ nAChR agonists (GTS-21, nicotine and AR-R17779) stimulate $\alpha 7$ nAChRs to elicit activation of CAP resulting in alleviated disease activity.^{5,8,11,12} Also, α -bgt and methyllycaconitine (specific $\alpha 7$ nAChR antagonists) can block $\alpha 7$ receptors and

deactivate the anti-inflammatory effect of the CAP in several inflammations.^{12,13} In addition, knockdown of $\alpha 7$ nAChR in mice with experimental arthritis increases production of proinflammatory cytokines, aggravates synovial inflammation and suppress the adaptive immunity by decreasing proliferative immune response.^{14,15} Several studies have shown that activation of the $\alpha 7$ inhibits NF- κ B nuclear translocation by interfering with I- κ B phosphorylation and NF- κ B transcriptional activity, meanwhile, activates the JAK2/STAT3 signalling pathway, leading to the repression of the inflammatory reactions.^{16,17} For example, in endotoxin-induced experimental sepsis, nicotine inhibited HMGB1 release from macrophages by suppressing the NF- κ B pathway via activating $\alpha 7$ nAChR.³ Upon the whole, all these data informed us about the CAP and $\alpha 7$ nAChR as crucial players with respect to the modulation of inflammation.

1.2 | Autoimmune rheumatic diseases

Autoimmune rheumatic diseases, consisting of multitudinous rare disorders, just name a few, such as rheumatoid arthritis (RA), osteoarthritis, spondyloarthropathy, systemic lupus erythematosus (SLE), primary Sjögren's syndrome (SS) and systemic sclerosis (SSc), are systemic disorders with multi-organ involvements. Not only do these disorders mainly make an impact on joints, which result in arthralgia and arthritis, but they also affect other systems, including the cardiovascular system, respiratory system and urinary system.¹⁸ As mentioned above, the CAP plays roles in the inflammatory response in many diseases, and it is no exception in autoimmune rheumatic diseases, with several signalling pathways activated in the courses of these diseases,¹⁹ the CAP has been extensively studied in recent years. This review will summarize the latest progress with the view of figuring out the role of the cholinergic pathway and how it interacts with inflammatory reactions in several autoimmune rheumatic diseases, and many advances are results from a wide range of experiments performed in vitro and in vivo.

2 | THE CAP AND RA

Rheumatoid arthritis is a chronic, aggressive, invasive and autoimmune-mediated disease, mainly manifested as synovitis and joint destruction, and its main pathological features are synovial cell hyperplasia, thickening of the lining layer, infiltration of various inflammatory cells, pannus formation, destruction of cartilages and bone tissues. Hyperplasia and invasion of the fibroblast-like synoviocytes (FLSs)—one of the main cells of the synovium lining layer—, generally speaking, play an important role in the initiation and continuation of chronic inflammatory processes in RA synovial tissue.^{20,21}

It is well known that cellular and humoral immunity are both engaged in the initiation and perpetuation of RA, while in the initiation of the disease process, cellular immunity appears to make more effects on synovitis and synovial proliferation. Several activities of cells are involved in this process, including differentiation and activation of T cells (mainly TH1 and TH17 cells) and B cells, macrophage migration, dendritic cell proliferation and, significantly, synovial cell hyperplasia. After being activated, these cells can secrete several pro-inflammatory cytokines which then mainly maintain an ongoing state of inflammation and destruction,²² so suppressing the differentiation and proliferation of these cells may be effective in slowing down the progression of RA and alleviating clinical symptoms.

The initiation of RA, mainly triggered by diverse mechanisms, including but not limited to exogenous antigens, infection and disturbed immune system, is more sophisticated while less remarkable than perpetuation in the whole process, and therefore, there were not many types of research on it and cell reactivities inside it, so does the effect of the CAP on it. Many pioneering studies have shown that $\alpha 7$ nAChR is detected in synovial lining cells and vessels in RA synovium and cultured synoviocytes^{23,24}; subsequently, several studies have demonstrated that $\alpha 7$ nAChR expressed not only on the membrane of immune cells mentioned above, as well as FLS.⁸ During the initiation and progression of RA, the expression of acetylcholine receptor is not impaired, but increased and its function is enhanced. In the joints and spleen of collagen-induced arthritis (CIA) rats, the expression of CHRNA7—the gene that encodes $\alpha 7$ nAChR²⁵—is significantly increased,²⁶ and by silencing this gene, it was found that the expression of inflammatory factors increased in RA FLSs¹⁴; which means that the activation of $\alpha 7$ nAChR in the inflammatory response increases to play an anti-inflammatory effect in RA. In the CIA mice with $\alpha 7$ nAChR knockout, the immune response decreased, especially the adaptive immunity, which was characterized by increased expression of pro-inflammatory cytokines, increased synovial inflammation and serious damage to the joint bone.^{14,15} To investigate the effect of the CAP on immune cells related to RA, Liu et al²⁷⁻³¹ have done several experiments in vivo and ex vivo with GTS-21 and nicotine, revealing that activated $\alpha 7$ nAChR attenuates the inflammatory response by affecting the activities of immune cells. They have found GTS-21 decreases dendritic cell infiltration into the synovium and downregulates the surface molecules CD80 and MHCII in DCs in the spleen of CIA mice, and it also inhibits Th1 differentiation in CD4+ T cells from patients with RA. As to nicotine, it suppresses Th1 and Th17 cell differentiation and macrophage migration by binding to $\alpha 7$ nAChR to activate the CAP, ameliorates the imbalance between Th1 and Th2 and promotes Th2 differentiation, which mitigates the severity of inflammation, leading to attenuation of arthritis in RA patients. In addition, a recent

study demonstrated that cholinergic agonists nicotine and AR-R17779 repress the proliferation of FLS and U937 cells (monocytic cell lines), as well the production of VEGF and MMPs expression by these cells³² (Figure 1).

The production of cytokines following differentiation and proliferation of immune cells and FLSs and the activation of related inflammatory pathways accelerate RA progression, resulting in persistent inflammation, excessive production of synovial fluid, pannus formation and cartilage destruction. A large number of cytokines, including TNF- α , IL-1, IL-6, IL-8 and other less significant altered inflammatory factors, are detected at the disease site in synovial tissue and fluid in RA, and among the identified cytokines in RA, interleukin-1 (IL-1) and tumour necrosis factor- α (TNF- α), particularly produced

by macrophages, are considered to induce synoviocytes-derived collagenase and prostaglandin E2 (PGE2), leading to accelerated bone resorption and degeneration of cartilage^{5,33}; GM-CSF (granulocyte-macrophage colony-stimulating factor), released by activated FLS, stimulates neutrophils attraction and activation.²⁴ As much as definitive evidence on biologic products directed against these cytokines has shown significant efficacy in the treatment of RA, studies focussed on the effect of the CAP on these cytokines occupy a very peculiar spot. ACh is well known to work on immune cells by activating several signalling pathways, as to whether it acts on immune cells directly, it remains inconclusive, but recent studies have shown it is capable of significantly reducing the production of cytokines. Ex vivo, ACh inhibits the release

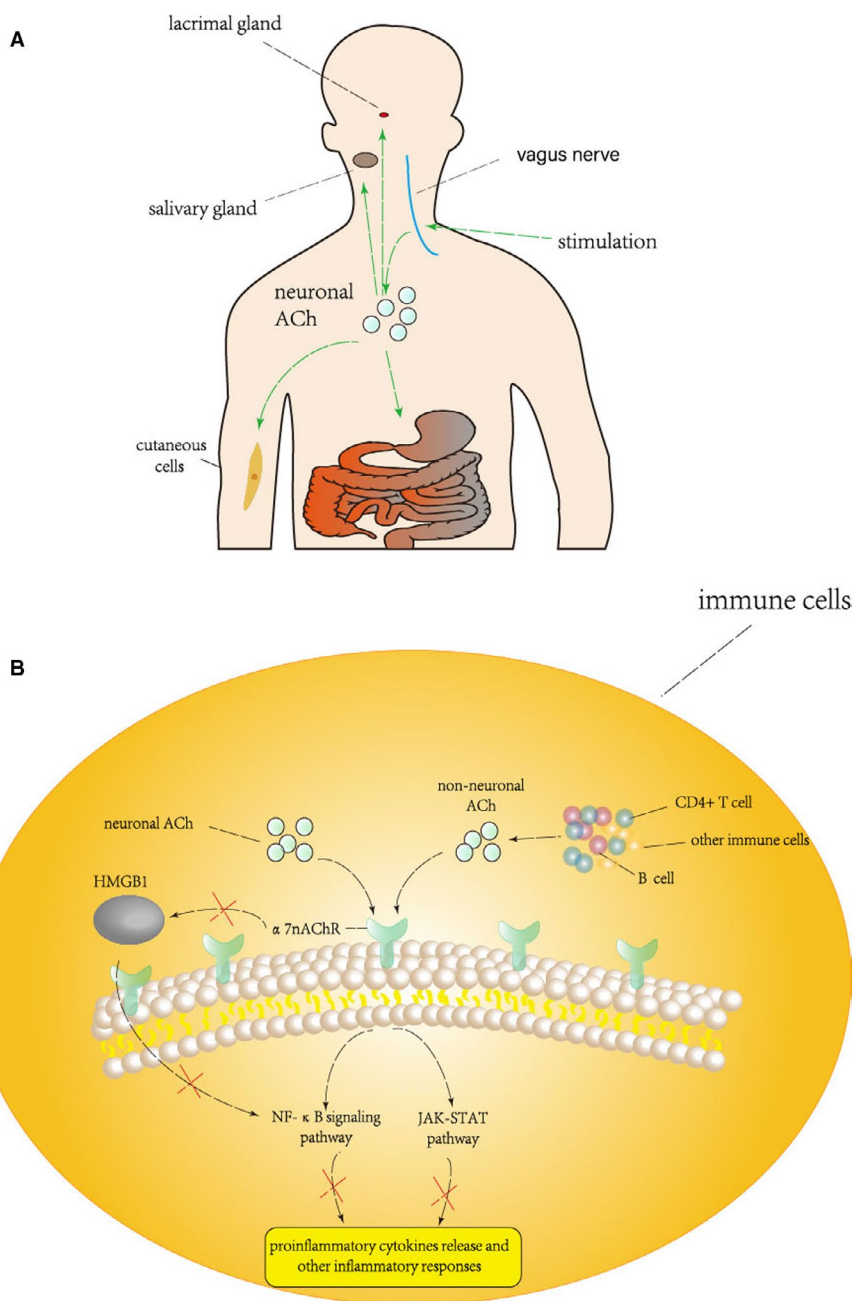


FIGURE 1 Possible pathways of the cholinergic anti-inflammatory pathway (CAP) in autoimmune rheumatic diseases. A, Stimulation of the vagus nerve can act on multiple organs and glands in several autoimmune diseases; B, The effect of the CAP is attributed to activation of $\alpha 7nAChR$; activated $\alpha 7nAChR$ could affect the activity of immune cells and signalling pathways, thereby inhibiting the release of pro-inflammatory cytokines. Abbreviations: ACh, acetylcholine; FLS, fibroblast-like synoviocytes; $\alpha 7nAChR$, nicotinic acetylcholine receptor $\alpha 7$ subunit; NF κ B, nuclear factor κ B; JAK, janus kinase; STAT, signal transducer and activator of transcription

of CXCL8 (IL-8), CCL2 (MCP-1), CCL3 (MIP-1 α) and CCL5 (RANTES) from IL-1-stimulated FLS with less IL-6 mRNA and protein expression.²⁴ $\alpha 7$ nAChR, more efficient perhaps in this case than of ACh, received more attention. In the FLS of RA patients cultured in vitro, $\alpha 7$ nAChR expressed at both gene and protein levels; and Ma van Maanen et al.³⁴ have observed a remarkable increase in production of IL-6, IL-8 and matrix metalloproteinase by adding a specific $\alpha 7$ nAChR shRNA to silence gene expression of $\alpha 7$ nAChR; besides, the data have shown specific $\alpha 7$ nAChR agonists reduced TNF- α induced IL-6 and IL-8 production by FLS; in addition, they also demonstrated nicotine and AR-R17779 (a specific $\alpha 7$ nAChR agonist) mitigated synovial inflammation via suppressing bone degradation and TNF- α expression.⁸ Furthermore, Zhou Y and his partners³⁵ have shown that nicotine, the effect of which is dose-dependent, reduced the protein and mRNA expression of IL-6 and IL-8 produced by FLS from patients with RA, then they investigated the effects of GTS-21 on the pathogenesis of RA in a collagen-induced arthritis model of mice, demonstrated that GTS-21 reduced the levels of serum TNF- α and IL-6, as a consequence of amelioration of the inflammatory response.²⁷ These results further emphasize the important role of $\alpha 7$ nAChR in inhibiting the release of inflammatory cytokines and regulating the inflammatory response in process of RA.

Several intracellular signalling transduction pathways that may interact with one another are known to be activated in both acute and chronic phases of RA to exert their anti-inflammatory effect, and here is knowledge in which the JAK-STAT pathway and NF- κ B signalling pathway do partake.^{19,36-38} NF- κ B activates in the pathogenesis of RA, especially in the synoviocytes, with which the most common effective form of NF- κ B—a heterodimer of p50 and p65—are associated. Phosphorylating I- κ B is necessary before NF- κ B migrates to the nucleus and binds to the promoter region of target genes to induce multiple inflammatory factors and modulate immune responses; in cultured FLS, the nuclear translocation of NF- κ B occurs rapidly after IL-1 or TNF stimulation yields induction of IL-6, IL-8, ICAM-1 and MMPs.^{39,40} In mouse macrophages, it has been shown in vitro and in vivo that the activation of $\alpha 7$ nAChR inhibits inflammation by interfering with I- κ B phosphorylation, suppressing NF- κ B nuclear translocation and activating the JAK2/STAT3 pathway.¹⁶ In CIA, ACh suppressed the activity of NF- κ B,²⁴ and nicotine inhibited NF- κ B translocation from the cytoplasm to the nucleus in synoviocytes³⁵; both of them can control the activation of the NF- κ B pathway through $\alpha 7$ nAChR to exert their anti-inflammatory effects. Nicotine mainly affects the JAK2/STAT3 pathway in the following three ways: interacting with STAT3 directly; binding to $\alpha 7$ nAChR to inhibit STAT3 phosphorylation, making STAT3 occupy NF- κ B subunits by taking the place of I- κ B, then avoiding NF- κ B to be activated; and nicotine also recruits JAK2, which is crucial

to phosphorylate STAT3 to $\alpha 7$ nAChR, leading the activation of the JAK2/STAT3 pathway.^{16,17,37,38} These three ways have been demonstrated in many mouse models of other diseases, but as for whether they work in RA or whether there are other connections between CAP and JAK2/STAT remains a mystery until now.

HMGB1 (High Mobility Group Box-1), a protein highly expressed in synovial tissues of RA patients and CIA mice, facilitates RA-FLS proliferation, migration, invasion and autophagy; reducing its expression can defer RA from aggravating.⁴¹⁻⁴³ HMGB1 activates the NF- κ B signalling pathway by binding to RAGE (advanced glycation end products) and TLR4 (Toll-like receptor4), leading to severe synovial inflammation.⁴³ Except as mentioned above ACh and nicotine interact with NF- κ B directly, they can also suppress HMGB1 release from macrophages, thereby reducing the activation of NF- κ B, and nicotine is more efficient than ACh in this aspect.³ ACh, nicotine and GTS-21 inhibit HMGB1 endocytosis, translocation and expression, but only the effect of nicotine was verified relating to RA in the inflamed joints of CIA mice without knowing the exact mechanisms.^{45,46}

In RA, the activity of the parasympathetic nervous system is decreased, especially the vagus nerve; deduced vagus nerve activity may enhance levels of cytokines and HMGB1, thus aggravating the severity of the disease.^{47,48} Vagotomy exaggerated inflammatory cell infiltration, cytokine release (particularly TNF- α and IL-6) and neutrophil recruitment towards joints, which plays a role in arthritis hyperalgesia and bone destruction in CIA mice, while the nicotine-pretreated group found the opposite.^{10,46,49-51} Stimulation of the vagus nerve not only reduced the severity of clinical symptoms and slowed disease progression in CIA and AIA, but also suppressed cytokine release and attenuated inflammation responses in patients with RA.^{10,52} Vagus nerve stimulation (VNS) reduces neutrophil recruitment, macrophage migration, cytokine release and active JAK2-STAT3 to control joint inflammation; most anti-inflammatory effects are due to activation of $\alpha 7$ nAChR.^{9,17,53} Results from pilot studies illustrated that non-invasive transcutaneous electrophysiological stimulation aimed at the vagus nerve, mainly cervical vagus nerve, could decrease cardiac vagal tone, reduce the severity of arthritis and inhibit pro-inflammatory cytokine release in RA patients and animal models of RA,^{14,52,54} and invasive VNS with implantable electrode could also suppress release of TNF in whole blood of patients with RA,⁵⁵ meaning that short-term electrical stimulation of the vagus nerve may be used as a newer and economical treatment for RA. However, the specific voltage, treatment cycle and adverse reactions are still unknown, and more experiments are needed to explore before large-scale clinical application. Vagus nerve suspension, an approach that can stimulate the vagus nerve for a long while, inhibits collagen antibody secretion, thereby alleviating arthritis in CIA mice.^{56,57} Parasympathetic integrity

is necessary to influence the inflammatory response; but the joints are not supplied directly by the vagus nerve as such, and contrary to the above conclusions, there was no significant correlation between the vagotomy and the incidence of future RA, so the anti-inflammatory action of CAP in RA is mainly attributed not to the vagus nerve itself, but other components, ACh and $\alpha 7$ nAChR in the pathway.^{10,58,59} The protein and mRNA of the choline acetyltransferase (ChAT)—the critical synthetic enzyme for ACh and a symbol of the functional activity of the cholinergic system—are detected in fibroblast-like and mononuclear-like cells in synovia tissue of RA patients via immunohistochemistry and situ hybridization,^{60,61} and with the knowledge that B cells, CD4+T cells, and other immune cells can produce ACh,^{62,63} maybe the vagus nerve is not necessary for the CAP; but as to whether the vagus nerves work in the CAP or RA, and what is the detailed mechanism of activity of CAP in RA is far from conclusive, and more studies are needed.

3 | THE CAP AND OSTEOARTHRITIS

Osteoarthritis (OA), which is a progressive disorder of weight-bearing joints (mainly metatarsophalangeal [MTP] joint of the hallux, the joints of hips, knees, and the lumbar and cervical spine), is characterized by cartilage degradation, subchondral bone remodelling, synovial inflammation, pain and disability.⁶⁴ The aetiology of OA remains unclear, and genetic and acquired risk factors such as age and weight may take their part in destroying the structure of cartilage, leading to the alteration in chondrocyte activity.⁶⁵ Altered chondrocytes can produce proteolytic enzymes (matrix metalloproteinases [MMPs]) to degrade collagen, which is the main component of the extracellular matrix, then cause loss of extracellular cartilage matrix in the initial phase.⁶⁶ Several chondrocyte-produced cytokines, which play a pivotal role in the initiation and progression of the pathologic process, including prostaglandins, IL-1 β , IL-6, TNF- α and nitric oxide (NO), can activate MMPs, thereby thinning out the matrix and gradually leading to loss of cartilage integrity.⁶⁷

Studies have observed that the autonomic nervous system (ANS) is involved in joint homeostasis and OA pathogenesis: the subchondral bone of OA patients has peripheral and cholinergic fibres, which innervate the synovium, trabecular bone and periosteum; and resident cells of osteoarticular tissue have receptors for ACh as chondrocytes express $\alpha 7$ nAChR on their surfaces.^{63,68,69} $\alpha 7$ nAChR was detected in chondrocytes of OA patients, but the increased expression was not as significant as that of patients with RA; meanwhile, mRNA expression of CHRFAM7A—which is capable of pruning $\alpha 7$ nAChR and associated with the cartilage degradation—was significantly higher than that of *Chrna7*,

indicating that functions of the CAP are impaired in OA, and this hypothesis was confirmed as prognosis of *Chrna7*^{-/-} mice that underwent meniscectomy was observably lower than that of normal control mice that got the same operation.⁶⁹ Moreover, the choline acetyltransferase (ChAT) is detected in OA synovium, which means that resident cells are capable of synthesizing and releasing ACh in situ without autonomic innervation.^{68,70} These data suggest that CAP may act on OA simultaneously through the vagus nerve and ACh, and then play a role in inhibiting OA development.

Studies on the relationship between CAP and osteoarthritis are not that much as on RA with only a few types of research focus on the effect of nicotine on OA. Epidemiological studies on whether smoking protects against OA revealed that the incidence of OA among smokers was lower. This hypothesis was confirmed that of non-smokers, indicating that smoking was a favourable factor for OA; and this protective effect may be derived from nicotine-sensitive cholinergic receptors.⁷¹⁻⁷⁵ Nicotine can protect cartilage from being damaged by acting on a variety of cells and tissue components and play a role in delaying the progression of OA. In the initial phase of OA, the metabolic balance of the extracellular matrix is disturbed by MMPs, manifesting as active chondrocyte metabolism and apoptosis of chondrocytes, which is mainly responsible for collagen breakdown and the progressive degeneration of cartilage.⁷⁶ At the right concentration, nicotine is capable of promoting chondrocyte proliferation and survival, as well as inhibiting apoptosis of chondrocytes mainly induced by IL-1 β in cell lines^{66,76}; besides, nicotine holds promise for enhancing the ability of human bone marrow stromal cells to differentiate into chondrocytes.⁷⁷ In another experiment, however, it has been found that nicotine disrupted the capacity of mesenchymal stem cells (MSCs) to differentiate into chondrocytes in vitro, and this effect of nicotine may act through the $\alpha 7$ nAChR as it had been elucidated that nAChRs are expressed on chondrocytes and MSCs.⁷⁸ The above two experimental results are inconsistent, so more experimental confirmations are needed.

Nicotine suppresses cartilage degradation in OA generally follows these two ways: increasing the amount of type II collagen directly, which is the main component of collagen in the extracellular matrix,^{66,77} and reducing the matrix metalloproteinase-9 (MMP-9), a potent enzyme produced by macrophage that aggravates inflammatory response by up-regulating the level of TNF- α and IL-1, resulting in the rate of collagen destruction.⁷⁹ The effects of nicotine may work through $\alpha 7$ nAChR, as this action can be inhibited by antagonists of the selective $\alpha 7$ nAChR; nicotine also downregulates the expression of MMP-9 by activating PI3K/Akt pathway and suppressing NF- κ B translocation.⁷⁹

In the early stage of OA, besides the unbalanced chondrocyte metabolic activities, the inflammatory response caused by a variety of cytokines also plays a promoting role in the

development of the disease; several cytokines are involved, among which mainly IL-1 β , TNF- α , IL-6, IL-15 and so on,⁸⁰ and IL-1 β can be used to induce apoptosis of chondrocytes.⁷⁶ In mouse models of OA, researchers found that nicotine attenuated the inflammatory response by reducing serum and synovial TNF- α levels, which may be mediated by increased $\alpha 7$ nAChR expression.⁸¹ The experiment of Alice and his colleagues has provided proof for this hypothesis. They found that CHRFAM7A in human OA increased the expression of MMP-3 and MMP-13. To further confirm the above hypothesis, they used chondrocytes from wild-type (WT) and $\alpha 7$ nAChR-deficient *Chrna7*^{-/-} mice to see whether there is a difference between the effects of nicotine on two different types of cells; and the results showed that nicotine reduced the inflammatory response of WT chondrocytes by suppressing the release of IL-6 and MMP in a dose-dependent manner, but this effect was not found in the *Chrna7*^{-/-} chondrocytes.⁶⁹ Several signalling pathways are involved in the pathological mechanism of OA, for example the NF- κ B pathway and PI3K/Akt pathway.⁸² In monosodium iodoacetate-induced osteoarthritis rats, nicotine inhibits activation of MAPK and NF- κ B pathway by binding to $\alpha 7$ nAChRs, as a result of decreased phosphorylation of p38, Erk1/2 and NF- κ B p65⁸³; and activation of the PI3K/Akt pathway is also needed for nicotine to prevent chondrocytes from apoptosis.⁷⁶

A recent trial conducted by Alice et al⁵⁵ involving 18 OA patients featured with erosive hands showed that auricular transcutaneous VNS reduced pain intensity of hands and the function of hands improved after a 4-week intervene, suggesting that VNS may be a new treatment for OA.

Given that there are still many gaps in studies on the relationship between CAP and OA, more studies in this area are needed in the future. However, the current data suggest that nicotine may be a therapeutic agent for OA, and VNS may work as well.

4 | THE CAP AND THE SPONDYLOARTHROPATHIES

The spondyloarthropathies (SpAs), defined as a group of arthritis with different clinical syndromes separately, include ankylosing spondylitis (AS), reactive arthritis (ReA), psoriatic arthritis (PsA), enteropathic arthritis (EA) and undifferentiated spondyloarthropathy (USpA). But these different subgroups have something in common that distinguishes them from RA and OA, SpAs usually occur in the spine, lower extremity and asymmetrical joints, particularly the sacroiliac joints; and enthesitis is the most cardinal clinical manifestation.⁸⁴ There are few studies on the relationship between CAP and SpAs, concerning only PsA and AS.

PsA has various symptoms, in addition to asymmetrical oligoarthritis; dactylitis, nail pitting and symmetrical

polyarthritis that mimics RA without rheumatoid nodules, psoriatic spondylitis and typical skin changes are also involved.⁸⁵ A meta-analysis showed that smoking increases the risk of psoriasis in the general population, but whether smoking is associated with the onset of PsA in psoriasis patients has different opinions: some studies have shown no significant link between smoking and the development of PsA, but others found smoking can reduce the incidence of PsA in the psoriasis population.^{85,86} This paradox may be explained by the following hypotheses: smoking decreased the expression of TNF- α , IL-1 and IL-8 in a way as it does among smokers with ulcerative colitis⁸⁷; the other possible way may through the CAP: nicotine, one of the most important components in cigarette, may suppress inflammatory response by binding to $\alpha 7$ nAChR as $\alpha 7$ nAChR were detected in synovial lining cells and vessels in PsA.^{23,86} However, these hypotheses have not been confirmed at present, and more studies are needed to explore whether smoking is associated with PSA and the possible mechanism of action.

As for AS, the main manifestation of which is low back pain accompanied by bilateral changes in the sacroiliac joints, there is no difference between smokers and non-smokers in manifestations and progression of AS.^{88,89}

5 | THE CAP AND SLE

Systemic lupus erythematosus is a multi-systemic autoimmune disease, involving the musculoskeletal system, cardiovascular system, urinary system, immune system, etc; and the main clinical manifestations are as follows: changes of skin and mucous membranes, especially the erythematous facial rash with a butterfly distribution across the malar and nasal prominences; arthralgias and non-erosive arthritis of joints on the hands; glomerular inflammation, necrosis; pericarditis and valve nodules.⁹⁰

Vagus nerve tone is reduced and autonomic nervous system function is damaged in SLE,⁹¹ and a double-blind study on 18 patients with SLE suggests that transcutaneous auricular VNS reduced pain, fatigue and joint score obviously compared to sham stimulation,⁹² but how does VNS produce this effect is unclear; besides the decreased vagus nerve activity, expression of $\alpha 7$ nAChR is also reduced, which aggravate the inflammatory response.⁹³ A current study found agents aimed at $\alpha 7$ nAChR have little effect on mice with late-stage SLE⁹⁴; this suggests that long-term receptor dysfunction may be irreversible and treatment should be carried out as soon as possible in the early stage of the disease. The expression of inflammatory cytokines such as TNF- α , IL-1 β and IL-6 in patients with lupus nephritis (LN)—a complication of SLE manifested as renal inflammation—is higher than in primary glomerulonephritis; $\alpha 7$ nAChR agonist decreases the level of these inflammatory factors, and $\alpha 7$ nAChR antagonist does

the opposite.⁹⁵ Reduced vagus nerve tone and depressed expression of $\alpha 7$ nAChR in monocytes in LN patients and SLE mice suggest that the function and integrity of CAP are impaired in SLE.^{93,96} A study found that unilateral vagotomy lowers blood pressure levels in SLE mice with hypertension, a manifestation that mainly comes from the progression of nephritis and also reduces renal inflammation; however, this conclusion is contradicted by the effect of vagotomy on increased inflammatory response in RA, more experiments are needed to determine whether other neuroimmune pathways compensate for the loss of a single vagus function.⁹⁶ Acetylcholinesterase (a serine hydrolase that hydrolyses ACh) inhibitor, which can directly increase efferent vagus nerve activity, also ameliorates splenic and renal inflammation and lowers blood pressure in SLE rats⁹⁷; this effect may work through CAP as reduced hydrolase can increase the activity of ACh, and the higher the activity of ACh, the more active CAP and the stronger the anti-inflammatory effect. In mice models of SLE, nicotine reduces the expression of cytokines, attenuates renal inflammation and decreases blood pressure, thereby preventing it from developing into hypertension.^{95,98}

Data above all indicate that CAP is impaired in SLE, every part of this pathway may exert its effect if be activated properly, and this may provide a new idea for the treatment of SLE.

6 | THE CAP AND SSc (SCLERODERMA)

Systemic sclerosis, also known as scleroderma, is an acquired rheumatic disease with high morbidity and mortality; and its typical symptoms are thickening and hardening of the skin, then evolving to damaged lungs, gastrointestinal tract and kidneys.⁹⁹

In SSc, the vagal nerve tone is reduced¹⁰⁰; a study found that transcutaneous electrical nerve stimulation (TENS) at gastrointestinal (GI) acupoints could ameliorate symptoms of upper GI, delay the disease process and enhance intestinal function, all of these effects may attribute to increased vagal activities induced by TENS.¹⁰¹ This study can be seen as a new research direction as it provides the possibility for the hypothesis that the VNS may improve the symptoms of SSc.

In the early stage of SSc, altered vascular function is the pivotal manifestation, including intimal proliferation in the small- and medium-sized arteries, luminal narrowing and obliteration.⁹⁹ ACh induces vasodilation in SSc patients, in which both vasodilation function and velocity were impaired.^{102,103} Smoking usually aggravates inflammation of blood vessels and gastrointestinal but can reduce skin lesions—fibrosis of which is the typical feature of SSc, and this unique effect may come from nicotine given the

following functions it has: interacting with immune cells directly; inhibiting releasing of cytokines; and increasing endothelial progenitor cells to form new vessels.¹⁰⁴ $\alpha 7$ nAChR can express in several cells of the skin,^{105,106} and among these cutaneous cells, the dermal fibroblast is the most important as it mediates the production of collagen and TGF- β and contraction of the surrounding extracellular matrix; $\alpha 7$ nAChR agonists can repress TGF- β -mediated responses in dermal fibroblasts,¹⁰⁵ perhaps this is another way in which nicotine can improve the symptoms of SSc. Apart from $\alpha 7$ nAChR, anti-ganglionic (nicotinic) acetylcholine receptor (gAChR) antibodies, particularly anti-gAChR $\alpha 3$ and $\beta 4$ antibodies, were also detected in the sera of SSc patients,¹⁰⁷ and the average level of anti-gAChR $\alpha 3$ Abs in the SSc patients with GI manifestations was much higher than that in the SSc without GI symptoms,¹⁰⁸ so the presence of gAChR antibodies may be the sole cause of SSc intestinal symptom and immune imbalance.

Besides nicotinic receptors, the parasympathetic nervous system also has muscarinic receptors including 5 muscarinic G protein-coupled receptors (five receptor subtypes [M(1)–M(5)]),¹⁰⁹ and muscarinic receptors are widely distributed in bronchial smooth muscle, gastrointestinal smooth muscle¹¹⁰; since it plays a less important role in CAP than nAChRs, it is only involved in SS and SSc. Gastrointestinal dysmotility in SSc may be due aberrant autoantibodies against the muscarinic-3 receptor (M3R), which is activated by ACh to regulate GI motility.¹¹¹ These autoantibodies hamper the binding of ACh to M3R¹¹¹ and also block indirect muscle response induced by electric field neural stimulation, then counteract its effects on smooth muscle, leading to less contraction of these muscles and impaired motor function.¹¹²

If further studies in this area provide evidence, activation of nicotinic and muscarinic receptors, or inhibition of muscarinic receptor antibody activity, could be used to treat SSc.

7 | THE CAP AND SS

Sjögren's syndrome is an autoimmune disease mainly involving salivary and lacrimal glands, and it is divided into primary and secondary two types—the main difference between the two types is whether they are complicated with connective tissue disease or not—and the main symptom is a painless enlargement of the salivary glands, which then develops into xerophthalmia (dry eyes) and (dry mouth).¹¹³

The M3 muscarinic acetylcholine receptor (M3R) plays a cardinal role in exerting an exocrine function in salivary and lacrimal glands.^{110,114} Previous studies have shown that deficiency of the M3 mAChR causes hyposalivation in mice,¹¹⁵ as to whether this conclusion is also valid in SS has not been studied in this regard, but many experiments have found the existence of antibody of M3R in SS. The autonomic nervous

system modulates saliva production through the functional M3R on acinar cells¹¹⁶; many experiments have detected circulating autoantibodies against the M3R in patients with both primary and secondary SS, and they compete with ACh to bind to M3R on acinar cells of human salivary gland, which in turn blocks the action of neurotransmitters, resulting in impaired secretory function.¹¹⁶⁻¹²¹ In addition to competing for M3R with ACh as mentioned earlier, the antibody also promotes the production of the inflammatory cytokine IL-17 by increasing the activation of circulating M3R-specific Th17 cells in the patients with pSS, resulting in exacerbated inflammatory response.¹²² The M3R is composed of four extracellular domains (the N-terminal, and the first, second and third extracellular loops),¹¹⁰ and only antibodies aimed at these extracellular domains can interfere with secretory function.¹²³ M3R antibodies affect the function of M3 in several ways, as follows: suppressing Ca^{2+} -influx in a human salivary gland cell line by autoantibodies against the second extracellular loop of M3R¹¹⁴; disturbing signal transduction, leading to translocation of the epithelial cell water channel¹²¹; inducing inflammatory factors release (especially PGE(2)) to inhibit Na^+/K^+ -ATPase activity^{124,125}; antibodies of M3R may reduce saliva production in one or more of these ways, or in other ways that have not yet been discovered.

The pioneering work of Fox and his colleagues¹²⁶ has demonstrated that muscarinic agonists (pilocarpine and cevimeline) stimulate both M1 and M3 receptors in salivary glands: by activating M3R, they promoted secretory function, and the effect of inhibiting inflammatory cytokine release and prevention of apoptosis is the result of binding to M1R; later studies have found that continuous administration of pilocarpine effectively can also induce M3R expression.¹²⁷ Other symptoms such as fatigue, anxiety and depression are associated with inflammatory responses, and non-invasive VNS reduced levels of cytokines including IL-6, IL-1 β and TNF- α in SS.¹²⁸ As mentioned above, receptor agonists and VNS may be used to treat SS.

8 | CONCLUSION AND FUTURE EXPECTATIONS

From the above-mentioned various in vivo and in vitro experimental results, it is shown that the relationship between the CAP and autoimmune rheumatic diseases is intricate but closely related; CAP plays an anti-inflammatory role in a variety of autoimmune rheumatic diseases, including RA, OA, SLE, SSc and SS—although the mechanisms of action vary—the remaining autoimmune diseases have not been studied as much, and some have not even been reviewed in a single article. In RA, all components of CAP do exert their effect: stimulation of the vagus nerve can reduce neutrophil recruitment, macrophage migration,

cytokine release and active JaK2-STAT3^{9,17,53}; ACh suppressed the activity of NF- κ B²⁴ and suppressed HMGB1 release from macrophages⁴⁴; but most anti-inflammatory effects of them are due to activation of α 7nAChR, so α 7nAChR is the most important part of CAP, as the activation of α 7nAChR can not only inhibit the FLSs proliferation, immune cell activation and macrophage migration³² and suppress the release of inflammatory factor such as IL-1, IL-6 and TNF- α , but also interact with multiple signalling pathways including NF- κ B and JAK2/STAT3 pathways,^{16,37} leading to alleviated inflammation responses, slowed disease progression and improved symptoms in patients with RA and models of animal. In OA and PsA, α 7nAChR is still the main action unit, but the mechanisms are different, nicotine, an α 7nAChR agonist, prevents cartilage from degradation by reducing the production of matrix metalloproteinase in OA⁷⁹; and its anti-inflammatory effect in PsA due to binding to α 7nAChR.^{23,86} The integrity of CAP is impaired in SLE, but every part of this pathway may exert its effect if be activated to attenuate renal inflammation and decrease blood pressure.^{95,98} In SSc and SS, muscarinic receptors (particularly M3R) serve a much larger purpose than other components of CAP, but autoantibodies against M3R are highly expressed in both diseases and reduce the contraction of smooth muscle in SSc¹¹² and secretory function in SS.¹²²

With such kinds of results from both human and animal studies, do CAP, assisted by other anti-inflammatory mechanisms, inhibits inflammatory reaction and alleviates clinical symptoms in autoimmune rheumatic diseases. Treatment regimens targeting CAP such as vagal nerve stimulation, administration of ACh and α 7nAChR agonists may work effectively in diseases mentioned above; while the findings are not exhaustive, these data are far more from conclusive, so more researches are still needed on the role of CAP in autoimmune diseases and the mechanism behind it.

ACKNOWLEDGEMENTS

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Jiaqi Lv wrote the manuscript and drew the pictures. Xiaoxiao Ji and Zhen Li edited the manuscript. Huiqin Hao involved in supervision.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in [repository name e.g. “figshare”] at [http://doi.org/\[doi\], reference number \[reference number\]](http://doi.org/[doi], reference number [reference number]).

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How to cite this article: Lv J, Ji X, Li Z, Hao H. The role of the cholinergic anti-inflammatory pathway in autoimmune rheumatic diseases. *Scand J Immunol*. 2021;94:e13092. <https://doi.org/10.1111/sji.13092>