Cholinergic nervous system as therapeutic approach for the treatment of arthritis
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General introduction
RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic immune-mediated inflammatory disease of unknown etiology, affecting about 1% of the adult population worldwide (1). RA has a 3-fold higher incidence in women compared to men and in addition to their impact on quality of life, the disease is associated with long-term morbidity and early mortality (2). RA is characterized by inflammation of the synovium leading to progressive destruction of cartilage and bone (3). Although all joints can be affected, small diarthrodial joints of hands, feet and knees are the most commonly affected joints. The primary manifestations are pain, swelling, and limited motion of joints.

After onset of clinical disease, the normally hypocellular synovial membrane becomes hyperplastic, comprising a superficial lining layer of synovial fibroblasts and macrophages, overlying an interstitial zone that contains a marked cellular infiltrate, which includes synovial fibroblasts, macrophages, mast cells, CD4+ T cells, CD8+ T cells, natural killer cells, B cells and plasma cells (4). The inflamed synovium invades adjacent cartilage and promotes articular destruction, which is mediated by the activities of osteoclasts, chondrocytes and synovial fibroblasts. Articular damage in turn probably generates a rich source of neo-antigens to promote further autoimmune reactivity. Cytokines are implicated in each phase of the pathogenesis of RA, by promoting autoimmunity, by maintaining chronic inflammatory synovitis, and by driving the destruction of adjacent joint tissue (5;6).

CAUSE AND PATHOGENESIS

Although the precise etiology of the disease remains elusive, there is strong evidence for autoimmunity, as several autoantibodies are associated with RA. Rheumatoid factor (RF) is detected in the majority of patients with established disease, and constitutes one of the American College of Rheumatology (ACR) classification criteria. Another group of autoantibodies, referred to as antibodies to citrullinated protein antigen (ACPA), was later shown to be more specific for RA. This group of antibodies targets epitopes in which arginine is converted by peptidylarginine deiminase into citrulline during a posttranslational modification. Joint destruction, comorbidities such as cardiovascular disease, and other extra-articular manifestations are all most prominent in the ACPA-positive subset of RA patients (7). Genetic studies have demonstrated that MHC class II genes, in particular HLA-DR-B1 alleles, appear to have a strong association with RA. This conserved sequence is commonly known as the shared epitope (SE) and in most studies the presence of the SE is associated with increased joint destruction. The best established environmental risk factor for rheumatoid arthritis is cigarette smoking (8). Smoking was shown in several studies to
be a risk factor mainly in the ACPA-positive but not in the ACPA-negative RA. The disease risk conferred by smoking was greatly enhanced in individuals carrying the HLA-DR-B1 SE (9).

These data, in particular the gene–environment interaction between smoking and the HLA-DR-B1 SE genes, indicate that smoking might trigger some rather specific immune reactions present in some but not all RA patients, and do so in the presence of certain genes, thereby potentially revealing a pathogenetic mechanism triggered by smoking and potentially other environmental triggers (10). Advances in research on the pathogenesis and underlying mechanisms of RA facilitate the development of new drugs. Increasing evidence has shown that the proinflammatory cytokine TNFα plays a key role in RA (11) and TNFα blockade has shown its potential as systemic therapy for RA patients and other inflammatory diseases like psoriatic arthritis, ankylosing spondylitis and Crohn’s disease (12). Although the introduction of anti-TNFα therapy has played a major role in improving many patient outcomes (12), there is still a need for the identification of new pathways involved in the modulation of inflammation in order to further increase efficacy, in particular in patients not responding to current therapies.

**RELATION BETWEEN NERVOUS SYSTEM AND ARTHRITIS**

Several observations point towards a neurological involvement in the etiology and pathology of RA. The first thought to neurological involvement in RA was in the beginning of last century because a significant improvement of arthritis after ganglionectomies was reported (13). Later on in the 60’s it was observed that epilepsy patients using phenytoin had a reduced instance of RA. In the 80’s a number of studies have shown positive results with phenytoin in RA. Nowadays, modern anti-epileptic drug, as pregabalin and gabapentin, are being evaluated in the management of arthritis (14).

Two components of the nervous system innervate the synovium: efferent sympathetic nervous and afferent sensory nervous system. The observation that suggests neurological involvement in RA is the symmetry and typical anatomical distribution of joints involved in RA. A number of studies and case reports have shown that denervation of the joint inhibits the development of arthritis. For example, a patient with a longstanding hemiplegia who subsequently developed arthritis on the non-hemiplegic side but not the hemiplegic side (15). In a second case, a patient developed arthritis mutilans in all digits of both hands with the exception of the left 4th finger, which had prior sensory denervation following traumatic nerve dissection. The nerve supply was further detailed using nerve conduction studies that confirmed the complete absence of sensory innervation in the left 4th digit (16).
Animal and human studies provide further evidence of the influence of the nervous system on synovial inflammation. Human RA synovium expresses markers of increased sensory innervation (substance P expression) while sympathetic nerve fibers are lost (17). This is in comparison with osteoarthritic synovium (18).

**CHOLINERGIC NERVOUS SYSTEM AS THERAPEUTIC APPROACH**

In recent years, the parasympathetic part of the nervous system and nicotinic acetylcholine receptors have been identified as crucial mediators of the inflammatory response. This concept is called “the cholinergic antiinflammatory pathway” and is mainly formed by the 10th cranial nerve, the vagus nerve. This nerve may exert anti-inflammatory effects by the release of its principal neurotransmitter acetylcholine (ACh). *In vitro* studies have shown that macrophages, activated by endotoxin, are effectively deactivated in the presence of ACh, characterized by a dose-dependent reduction in the release of a series of proinflammatory cytokines (19). The antiinflammatory effects of ACh are mediated by nicotinic acetylcholine receptors, and in particular by the α7 subunit of nicotinic acetylcholine receptors (α7nAChR) (20). Besides expression on brain and neuronal cells, the α7nAChR is also expressed by macrophages and other immune cells (20-22) and recently it has been shown that cultured fibroblast-like synoviocytes (FLS) and synovial tissue biopsies from RA patients express α7nAChR (23).

In several studies it was demonstrated that activation of the cholinergic anti-inflammatory pathway ameliorates disease in animal models of endotoxemic shock (19), ischemia-reperfusion injury (24), hypovolemic-hemorrhagic shock (25), peritonitis (26), pancreatitis (27), experimentally induced ileus (28) and carrageenan-induced paw inflammation in rats (29). However, vagus nerve stimulation in α7-deficient mice failed to reduce plasma TNFα levels during endotoxemia (8).

**OUTLINE OF THIS THESIS**

Chapter 2 provides a perspective of the role of the cholinergic antiinflammatory pathway and the therapeutic potential of modulating this pathway in RA. In this chapter the role of the cholinergic pathway in several animal models of inflammatory diseases is described. Furthermore, it describes the structural and functional aspects of the α7nAChR and its duplicate variant, discusses vagus nerve stimulation (VNS) and pharmacological activation of α7nAChR as novel strategies for the treatment of RA and the possible underlying mechanisms.
The objective of the study in Chapter 3 was to obtain insight into the role of the cholinergic anti-inflammatory pathway in arthritis by manipulating this pathway in vivo. Therefore, we studied the effect on collagen-induced arthritis (CIA) in mice either by inhibition of the cholinergic anti-inflammatory pathway using unilateral cervical vagotomy or by stimulation of this pathway by administration of nicotine to the drinking water. In a separate study, CIA was induced in mice in which the peripheral part of this pathway was stimulated with intraperitoneally injected nicotine or the highly selective α7nAChR agonist AR-R17779.

In Chapter 4, we further investigated the role of the cholinergic antiinflammatory pathway in CIA, and more specifically delineated the role of α7nAChR herein. For this we compared clinical signs and symptoms of arthritis and onset and incidence of disease in α7nAChR deficient (α7nAChR−/−) and wild-type littermate mice (WT) in the CIA model. After sacrificing, we compared bone destruction, synovial inflammation, systemic proinflammatory cytokines and IgG2a:IgG1 ratio between the α7nAChR−/− mice and littermates. Since some reports indicate that the spleen may play a critical role in exerting the antiinflammatory effects of the cholinergic pathway, we analyzed the antigen-specific production of Th1- and Th2-associated cytokines by spleen cells.

The first results of stimulation of the cholinergic antiinflammatory pathway by pharmacologic activation of the α7nAChR were shown in chapter 3. In these CIA experiments the agonist AR-R17779 was used. The objective of the study in Chapter 5 was to investigate the pharmacological and functional profile of two novel compounds, CTI-15311 and CTI-15072, with different effects on ion channel activity and investigated the therapeutic effects of both compounds on experimental arthritis. Both agonists poorly pass the blood-brain barrier and are more specific for α7nAChR than AR-R17779. The agonists were delivered by oral gavage before the onset of arthritis or during established arthritis.

Taken together, the results described in Chapters 3 through 5 support the notion that the cholinergic antiinflammatory pathway plays a role in inflammatory arthritis in mice. It is known that CIA is a reliable animal model to predict the clinical efficacy of new therapeutic agents in human RA (30). This leads to the hypothesis that a feasible therapeutic strategy would be to stimulate the cholinergic antiinflammatory pathway by pharmacologic activation of the α7nAChR in the human situation. To be able to understand the effects of novel treatments in human RA, it is important to evaluate the effects on the primary site of inflammation, the synovium. In Chapter 6, experiments are described focusing on the synovial tissue and FLS of RA patients. It is known that the α7nAChR is expressed by various immune cells among which monocytes (31), macrophages (19;32), T and B lymphocytes (33;34), and dendritic cells (35). Accumulating evidence suggests that FLS play a major role in the initiation and perpetuation...
of the chronic inflammatory process in RA synovial tissue (4;36;37). Therefore, we investigated the expression of a7nAChR in synovial tissue and FLS of RA patients. Moreover, we performed functional studies by evaluating the effects of specific a7nAChR agonists on the production of key proinflammatory cytokines and chemokines by activated RA FLS.

Chapter 7 is dedicated to the summary and general discussion of the main findings of the studies presented in chapter 2-6 in light of the recent literature.
REFERENCES


Chapter 1


