UvA-DARE (Digital Academic Repository)

Role of the cholinergic nervous system in rheumatoid arthritis: aggravation of arthritis in nicotinic acetylcholine receptor alpha7 subunit gene knockout mice

van Maanen, M.A.; Stoof, S.P.; Larosa, G.J.; Vervoordeldonk, M.J.; Tak, P.P.

DOI

[10.1136/ard.2009.118554](https://doi.org/10.1136/ard.2009.118554)

Publication date

2010

Document Version

Final published version

Published in

Annals of the Rheumatic Diseases

[Link to publication](#)

Citation for published version (APA):

van Maanen, M. A., Stoof, S. P., Larosa, G. J., Vervoordeldonk, M. J., & Tak, P. P. (2010). Role of the cholinergic nervous system in rheumatoid arthritis: aggravation of arthritis in nicotinic acetylcholine receptor alpha7 subunit gene knockout mice. *Annals of the Rheumatic Diseases*, 69(9), 1717-1723. <https://doi.org/10.1136/ard.2009.118554>

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (<https://dare.uva.nl>)

Role of the cholinergic nervous system in rheumatoid arthritis: aggravation of arthritis in nicotinic acetylcholine receptor $\alpha 7$ subunit gene knockout mice

Marjolein A van Maanen,¹ Susanne P Stoof,^{1,2} Gregory J LaRosa,^{3,4} Margriet J Vervoordeldonk,^{1,2} Paul P Tak¹

► Additional data are published online only. To view these files please visit the journal online (<http://ard.bmj.com>).

¹Division of Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam, Amsterdam, The Netherlands

²Academic Medical Center/University of Amsterdam, Arthrogen BV, Amsterdam, The Netherlands

³Cornerstone Therapeutics Inc, Cary, North Carolina, USA

⁴Bikam Pharmaceuticals, Cambridge, Massachusetts, USA

Correspondence to

Dr P P Tak, Division of Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam, F4-105, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands; p.p.tak@amc.uva.nl

Accepted 14 February 2010
MJV and PPT contributed equally to this work

ABSTRACT

Background The $\alpha 7$ subunit of nicotinic acetylcholine receptors ($\alpha 7$ nAChR) can negatively regulate the synthesis and release of proinflammatory cytokines by macrophages and fibroblast-like synoviocytes in vitro. In addition, stimulation of the $\alpha 7$ nAChR can reduce the severity of arthritis in murine collagen-induced arthritis (CIA).

Objective To provide more insight into the role of the $\alpha 7$ nAChR in the pathogenesis of arthritis by investigating the effect of the absence of $\alpha 7$ nAChR in CIA in $\alpha 7$ -deficient ($\alpha 7$ nAChR^{-/-}) compared with wild-type (WT) mice.

Methods CIA was induced in $\alpha 7$ nAChR^{-/-} and WT littermate mice at day 0 by immunisation with chicken collagen type II (cCII) followed by a booster injection with cCII on day 20. Mice were killed on day 44 or day 63 and arthritis activity as well as radiological and histological damage were scored. The effects on the immune response were evaluated by measurement of antigen-specific antibodies and cytokines, and evaluation of the effects on antigen-specific stimulated spleen cells.

Results In $\alpha 7$ nAChR^{-/-} mice a significant increase in the incidence and severity of arthritis as well as increased synovial inflammation and joint destruction were seen. Exacerbation of CIA was associated with elevated systemic proinflammatory cytokines and enhanced T-helper cell 1 (Th1)-cytokine and tumour necrosis factor α production by spleen cells. Moreover, a specific decrease in the collagen-specific 'Th1-associated' IgG2a response was seen, whereas IgG1 titres were unaffected.

Conclusions The results presented here indicate that immune cell function in a model of rheumatoid arthritis is regulated by the cholinergic system and, at least in part, mediated by the $\alpha 7$ nAChR.

INTRODUCTION

The nervous system, via an inflammatory reflex of the vagus nerve, has an important role in limiting inflammatory responses, a concept referred to as the 'cholinergic anti-inflammatory pathway'.¹⁻⁴ This pathway provides the host with a powerful mechanism for counteracting excessive inflammation in a very fast, discrete and localised way. Disruption of the vagus nerve by vagotomy exaggerated inflammation in various animal models: vagotomy enhanced systemic tumour necrosis factor α (TNF α) production and accelerated shock development in the experimental endotoxaemia model in rats,⁵ and enhanced the local and systemic inflammation accompanying bacterial peritonitis⁶ and cerulean-induced acute pancreatitis in mice.⁷

On the other hand, electrical stimulation of the vagus nerve resulted in inhibition of inflammation: it downregulated TNF α production and protected endotoxaemic rats from hypotension,⁵ it inhibited the acute inflammatory response to hypovolaemic haemorrhagic shock,⁸ and diminished intestinal inflammation during experimentally induced ileus in mice.⁹

Acetylcholine (ACh), the principal neurotransmitter of the vagus nerve, is a key mediator of this cholinergic anti-inflammatory pathway. ACh interacts with members of the nicotinic acetylcholine receptor (nAChR) family, and in particular the $\alpha 7$ subunit of nicotinic acetylcholine receptor ($\alpha 7$ nAChR). Nicotine exerts anti-inflammatory effects on macrophages that can be counteracted by selective $\alpha 7$ antagonists,^{1 2 10} and selective nAChR agonists have proved effective in reducing macrophage cytokine production and inflammation in animal models of pancreatitis,⁷ experimentally induced ileus in mice⁹ and improving survival in mice challenged with lipopolysaccharide.^{11 12} Accordingly, electrical vagus stimulation in $\alpha 7$ nAChR knockout ($\alpha 7$ nAChR^{-/-}) mice failed to reduce serum TNF α levels during endotoxaemia¹⁰ and splenocytes, and peritoneal macrophages derived from these mice were shown to be insensitive to the cytokine-inhibiting effects of cholinergic agonists.^{10 13} It was recently shown that $\alpha 7$ nAChR^{-/-} mice were not protected from renal ischaemia/reperfusion injury by nicotine pretreatment in comparison with successful pretreatment in littermate wild-type (WT) mice.¹⁴ Moreover, another recent study showed that deficiency of the $\alpha 7$ subunit is associated with an accelerated clearance of *Escherichia coli* after intraperitoneal infection, preceded by a faster recruitment of neutrophils.¹⁵

Recently, we have demonstrated that the cholinergic anti-inflammatory pathway has an important role in limiting inflammatory responses in collagen-induced arthritis (CIA), a well-known animal model of rheumatoid arthritis (RA).¹⁶ Vagotomy aggravated, and oral administration and intraperitoneally injected nicotine ameliorated, clinical arthritis and bone degradation and reduced TNF α expression in synovial tissue. The effect of AR-R17779, a selective $\alpha 7$ nAChR agonist,¹⁷ ameliorated clinical signs and symptoms of arthritis and was more potent than nicotine. The reduction of arthritis was associated with delayed onset of the disease as well as a protective effect against joint destruction and a reduction of TNF α level in both serum and synovial tissue. Consistent with these results,

Extended report

electrical and pharmacological stimulation of the vagus nerve results in decreased carrageenan-induced paw inflammation in rats.¹⁸ Thereby, several studies have shown that the sympathetic nervous system has an anti-inflammatory role in late arthritis.¹⁹ The relevance for human RA is underlined by recent studies, showing the expression of the $\alpha 7$ nAChR in the inflamed synovium of patients with RA.^{20 21}

CIA is restricted to mice bearing the major histocompatibility complex class II H-2^a or H-2ⁱ, but not H-2^b, haplotypes.^{22 23} Therefore, strains of mice on a C57BL/6 background (H-2^b) have been thought to be resistant to CIA, and most of the genetically modified strains of mice are on a C57BL/6 background. However, recently it has been demonstrated that C57BL/6 mice could be susceptible to CIA using chicken collagen type II (cCII).^{24–26} This implies that the CIA model can be directly applied to strains deficient in certain genes, which might be involved in the pathogenesis of RA. To provide more insight into the role of the $\alpha 7$ nAChR in the pathogenesis of arthritis, we compared CIA in $\alpha 7$ nAChR^{-/-} and wild-type (WT) mice and evaluated the clinical expression of the disease and the effects of the $\alpha 7$ nAChR on the immune response in this model of RA.

MATERIALS AND METHODS

Animals

Mice deficient for the $\alpha 7$ subunit gene of the $\alpha 7$ nAChR ($\alpha 7$ nAChR^{-/-})²⁷ and WT littermates were bred on a C57BL/6 background by Charles River Diagnostics (Wilmington, Massachusetts, USA). Male and female mice about 10–12 weeks old were used for the experiments. They were housed under specific pathogen-free conditions at the animal facility of the Academic Medical Center/University of Amsterdam (Amsterdam, The Netherlands). Feeding was ad libitum. The Institutional Animal Care and Use Committee of the Academic Medical Center approved all experiments.

Induction and assessment of CIA

Mice were immunised with CIA as previously described.²⁴ Briefly, cCII (Sigma Chemical Co, St Louis, Missouri, USA) was dissolved in 0.1 M acetic acid to a concentration of 2.0 mg/ml by overnight rotation at 4°C and mixed with an equal volume of Freund's complete adjuvant (2.5 mg/ml of *Mycobacterium tuberculosis*; Chondrex, Redmond, Washington, District of Columbia, USA). The mice were immunised intradermally at the base of the tail with 100 μ l of emulsion on day 0. The same injection was repeated on day 20.

The severity of the arthritis was assessed using an established semiquantitative scoring system of 0–4 where 0=normal, 1=mild swelling, 2=moderate swelling, 3=swelling of all joints and 4=joint distortion and/or rigidity and dysfunction.²⁸ The cumulative score for all four paws of each mouse (maximum possible score 16) was used as the arthritis score to represent overall disease severity and progression in an animal. For the evaluation of incidence, mice were considered to have arthritis if the clinical arthritis score increased by at least one point for two consecutive days.

Study design and evaluation of arthritis activity

In study 1, we evaluated the role of $\alpha 7$ nAChR in CIA using $\alpha 7$ nAChR^{-/-} and WT mice (n=20 per group; 10 male, 10 female). Mice were killed on day 63 and hind paws and serum of all mice were used for further analysis. In study 2, CIA was performed in $\alpha 7$ nAChR^{-/-} (n=19; 5 male, 14 female) and WT mice (n=16; 5 male, 11 female) and mice were killed on day 44, which

represents the more acute phase of the disease. Hind paws, serum and spleens were used for further analysis. In both studies mice were inspected three times a week for signs of arthritis by two independent observers who were not aware of the genetic background.

Radiological analysis

The knee joint of the hind paws was used for radiographic evaluation. Joint destruction was scored on a scale from 0 to 4, where 0=no damage, 1=demineralisation, 2=one or two erosions, 3=severe erosions and 4=complete destruction of the joints. The radiographs were scored by two independent observers (MAvM and DM Gerlag) in a blinded manner; minor differences in scoring between the observers were resolved by mutual agreement.

Histological analysis

Hind paws were fixed for 24 h in 10% buffered formalin and decalcified in 15% EDTA. The paws were then embedded in paraffin, and serial 5 μ m sagittal sections of whole hind paws were cut and stained with H&E. Three independent observers (MAvM, SPS and MJV) assessed the tissue for the degree of synovitis and cartilage degradation by microscopic evaluation, under blinded conditions, as described previously.^{29 30} Synovitis and cartilage degradation in the knee joints were graded on a scale of 0 (no inflammation) to 3 (severely inflamed joint) based on the extent of infiltration by inflammatory cells into the synovium. Sections were also stained with safranin O-fast green to determine depletion of proteoglycans. Safranin O staining was scored with a semiquantitative scoring system of 0 (no loss of proteoglycans) to 3 (complete loss of proteoglycans) in the knee joint.²⁹

Measurement of antigen-specific antibodies in serum

Serum levels of antibodies against cCII were measured by ELISA. A 96-well plate was coated with cCII (5 μ g/ml; Chondrex) and incubated overnight at 4°C. Non-specific binding was blocked with phosphate-buffered saline containing 1% bovine serum albumin for 1 h at room temperature. Serial dilutions of standard (mouse anti-type II collagen IgG1 or IgG2a) antibody solution (10 ng/ml, Chondrex) and serum samples were incubated for 1 h at room temperature, followed by the addition of horseradish peroxidase-conjugated rat anti-mouse IgG1 or IgG2a (BD Biosciences, San Jose, California, USA). After 1 h of incubation at room temperature, tetramethylbenzidine substrate was added and the optical density at 450/540 nm was measured using a microplate reader.

Isolation and culture of splenic cell populations

In study 2, the spleen was extracted at the time of death and single-cell suspensions were prepared in complete medium (RPMI 1640 supplemented with 10% fetal calf serum). Splenocytes were cultured at 4×10^6 cells per well in duplicate, in the absence or presence of cCII (25 μ g/ml) for 48 h. Supernatants were harvested.

Assays

Levels of TNF α , interleukin 6 (IL-6), monocyte chemoattractant protein 1 (MCP-1), IL-12 p70, interferon γ (IFN γ) and IL-10 in serum and supernatants of splenic cell cultures were determined using a commercially available cytometric bead array multiplex assay (BD Biosciences).

Statistical analysis

To evaluate the effects of $\alpha 7$ nAChR, we determined the change in clinical arthritis scores in each mouse from day 20 until the end of the experiment. Areas under the curve for the change in arthritis scores were calculated. The significance of the differences in mean changes in scores (clinical, radiological, histological and immunohistochemical) between groups was determined by the Mann–Whitney U test (SPSS version 12.0.2; SPSS, Chicago, Illinois, USA). Incidence was compared using Kaplan–Meier survival analysis (GraphPad Prism version 4.03; GraphPad Software, San Diego, California, USA). Cytokine levels were compared by Mann–Whitney U test. *p* Values <0.05 were considered statistically significant.

RESULTS

Aggravation of clinical arthritis and increased incidence of disease in $\alpha 7$ nAChR^{-/-} mice

In both studies we evaluated the role of $\alpha 7$ nAChR in CIA. In agreement with the original description of these mice,²⁷ mice lacking the $\alpha 7$ subunit developed normally and showed no gross anatomical defects or any gross phenotypic abnormalities. In study 1, all mice were killed on day 63, the point at which arthritis has reached the chronic phase. An aggravation of clinical signs of arthritis was seen in the arthritic $\alpha 7$ nAChR^{-/-} mice compared with arthritic WT mice (*p*<0.05) (figure 1A,B). In addition, $\alpha 7$ nAChR^{-/-} mice showed an accelerated onset of disease and a significantly higher disease incidence (*p*<0.05) (figure 1C).

Increased bone destruction and cartilage degradation in $\alpha 7$ nAChR^{-/-} mice in the chronic phase of the disease

To examine the effects of $\alpha 7$ nAChR on bone degradation, radiographs of knee joints obtained at the end of the experiment on

day 63 were evaluated. As seen in figure 2A,B, joint destruction was significantly increased in $\alpha 7$ nAChR^{-/-} mice (*p*<0.05). Next, cartilage degradation was assessed by safranin O-fast green staining, a method for detecting depletion of proteoglycans. Proteoglycan loss was significantly higher in the knee joints of $\alpha 7$ nAChR^{-/-} mice (*p*<0.05) (figure 2C).

In addition, analysis of synovial inflammation was assessed by H&E staining of knee joints (figure 3A). Histological scoring showed a significant increase in cartilage degradation in $\alpha 7$ nAChR^{-/-} mice compared with WT mice (figure 3B) (*p*<0.05). Similarly, $\alpha 7$ nAChR^{-/-} mice showed a non-significant trend towards increased cellular influx into the synovium at day 63 (data not shown).

Increased incidence and aggravated severity of arthritis and synovial inflammation in $\alpha 7$ nAChR^{-/-} mice in the acute phase of the disease

In study 2, in order to investigate the effects of $\alpha 7$ nAChR in the more acute phase of the disease, we killed all mice on day 44. Similar to the results in study 1, the clinical arthritis score was increased in $\alpha 7$ nAChR^{-/-} mice compared with WT littermates (*p*<0.005) (figure 4A,B). In addition, $\alpha 7$ nAChR^{-/-} mice showed an accelerated onset of disease and a significantly higher disease incidence (*p*<0.05) (figure 4C). Synovial inflammation was assessed by H&E staining of the knee joints. There was a significant increase in inflammatory cell infiltration in $\alpha 7$ nAChR^{-/-} mice, killed in the acute phase of the disease (figure 4D) (*p*<0.05).

Increased serum levels of MCP-1 and TNF α in $\alpha 7$ nAChR^{-/-} mice

To investigate the effect of the $\alpha 7$ nAChR on systemic cytokine concentrations in the acute phase of the disease, we assessed serum levels of different proinflammatory cytokines

Figure 1 Exacerbation of arthritis in $\alpha 7$ subunit of nicotinic acetylcholine receptor ($\alpha 7$ nAChR)-deficient ($\alpha 7$ nAChR^{-/-}) versus wild-type (WT) mice (*n*=20 per group). Arthritis was induced in mice by immunisation with type II collagen, and evaluated three times a week by two independent observers, under blinded conditions. (A) $\alpha 7$ nAChR^{-/-} mice showed an increase in arthritis scores compared with WT mice. Only mice with arthritis are shown. (B) Area under the curve (AUC) (days 20–63) for arthritic mice was significantly increased in $\alpha 7$ nAChR^{-/-} mice versus WT mice. **p*<0.05. (C) $\alpha 7$ nAChR^{-/-} mice showed a significant increase in disease incidence compared with the control group. **p*<0.05.

Extended report

Figure 2 Aggravation of bone degradation and proteoglycan depletion in murine collagen-induced arthritis in $\alpha 7$ subunit of nicotinic acetylcholine receptor ($\alpha 7nAChR$)-deficient ($\alpha 7nAChR^{-/-}$) mice compared with wild-type (WT) mice (n=20 per group). (A) Representative radiographs of the knee joints of an $\alpha 7nAChR^{-/-}$ mouse and a WT mouse. (B) Semiquantitative scores for joint destruction. Joint destruction was significantly increased in $\alpha 7nAChR^{-/-}$ mice. (C) Semiquantitative scores for proteoglycan depletion assessed by safranin O-fast green staining. Proteoglycan loss was significantly higher in the knee joints of $\alpha 7nAChR^{-/-}$ mice. Values are the mean ± SEM. *p<0.05.

by cytometric bead array multiplex assay. In the serum of $\alpha 7nAChR^{-/-}$ mice killed on day 44 the levels of MCP-1 and TNF α were significantly increased (p<0.05) (figure 5A,B). In addition, there was a non-significant trend towards increased IL-6 serum levels in $\alpha 7nAChR^{-/-}$ mice (data not shown). The serum levels of IL-12 p70, IFN γ and IL-10 were not significantly different between $\alpha 7nAChR^{-/-}$ and WT mice (data not shown).

Enhanced T-helper cell 1 (Th1) response in $\alpha 7nAChR^{-/-}$ mice

In study 2, we investigated the mechanisms underlying the severity of CIA. It has previously been shown that B-cell-deficient mice are resistant to CIA, indicating that collagen-specific antibodies are crucial for disease induction.³¹ Because the severity of CIA is reflected by the switch from a Th1 to a Th2 response,³² we measured the levels of IgG2a and IgG1 anti-CII antibodies in the serum, collected on day 44. The concentration of CII-specific IgG1 antibody was similar in $\alpha 7nAChR^{-/-}$ and WT mice, whereas the concentration of CII-specific IgG2a was increased in the $\alpha 7nAChR^{-/-}$ mice. This resulted in a markedly increased IgG2a:IgG1 ratio (p<0.05) (figure 5C).

Since switching towards the IgG2a isotype is strongly associated with a typical Th1 response in mice, and some reports indicate that the spleen may have a critical role in exerting the anti-inflammatory effects of the cholinergic pathway,^{13 33} we analysed the antigen-specific production of Th1-associated cytokine IFN γ and the Th2-associated cytokine

Figure 3 Synovial inflammation is increased in $\alpha 7$ subunit of nicotinic acetylcholine receptor-deficient ($\alpha 7nAChR^{-/-}$) mice in collagen-induced arthritis (n=20 per group). (A) Representative knee joint sections of an $\alpha 7nAChR^{-/-}$ mouse and a wild-type (WT) mouse. The $\alpha 7nAChR^{-/-}$ mice exhibited pronounced synovial inflammation (H&E stained; original magnification $\times 100$). (B) Semiquantitative scores for cartilage degradation in the knee joint. Cartilage degradation was significantly increased in $\alpha 7nAChR^{-/-}$ mice compared with WT mice. Values are the mean ± SEM. *p<0.05.

IL-10 by splenocytes. As shown in figure 6A, splenocytes from $\alpha 7nAChR^{-/-}$ mice produced significantly higher levels of IFN γ , whereas the level of IL-10 was not altered. Moreover, the production of the proinflammatory cytokines TNF α and IL-6, which both play a very important role in CIA,^{34 35} was increased in splenocytes from $\alpha 7nAChR^{-/-}$ mice (p<0.05) (figure 6B),

Figure 4 Exacerbation of arthritis in $\alpha 7$ nAChR deficient ($\alpha 7^{-/-}$) mice (n=19) versus wild-type (WT) mice (n=16) in the acute phase of disease. (A) The $\alpha 7$ subunit of nicotinic acetylcholine receptor deficient ($\alpha 7$ nAChR $^{-/-}$) mice showed an increase in arthritis scores compared with WT mice. (B) Area under the curve (AUC) (days 20–44) was significantly increased in $\alpha 7$ nAChR $^{-/-}$ mice versus WT mice. **p<0.005. (C) $\alpha 7$ nAChR $^{-/-}$ mice showed an accelerated onset of disease and a significantly higher disease incidence than the control group. *p<0.05. (D) Synovial inflammation, assessed by H&E staining of the knee joints, showed a significant increase in inflammatory cell infiltration in $\alpha 7$ nAChR $^{-/-}$ mice, killed in the acute phase of the disease. *p<0.05.

Figure 5 Serum levels of different proinflammatory cytokines were assessed to evaluate the effect of $\alpha 7$ subunit of nicotinic acetylcholine receptor ($\alpha 7$ nAChR) on systemic cytokine concentrations in the acute phase of the disease. (A,B) In the serum of $\alpha 7$ subunit of nicotinic acetylcholine receptor deficient ($\alpha 7$ nAChR $^{-/-}$) mice (n=17), killed on day 44, the levels of monocyte chemoattractant protein 1 (MCP-1) and tumour necrosis factor α (TNF α) were significantly increased compared with wild-type (WT) mice (n=17). Values are the mean $\pm$ SEM. *p<0.05. (C) The levels of IgG2a and IgG1 anti-CII antibodies in the serum were measured, collected on day 44. The concentration of CII-specific IgG1 antibody was similar in $\alpha 7$ nAChR $^{-/-}$ and WT mice, whereas the concentration of CII-specific IgG2a was increased in the $\alpha 7$ nAChR $^{-/-}$ mice. This resulted in a markedly increased IgG2a:IgG1 ratio. *p<0.05.

whereas the production of IL-12 p70 and MCP-1 was not significantly different between splenocytes of $\alpha 7$ nAChR $^{-/-}$ mice and WT littermates (data not shown).

DISCUSSION

The results presented in this paper show that mice deficient for $\alpha 7$ nAChR develop a marked increase in clinical arthritis scores

in both the acute and the chronic phase of the disease. This was accompanied by an increased incidence of arthritis, synovial inflammation, joint destruction and elevated levels of systemic proinflammatory cytokines. Of interest, $\alpha 7$ nAChR $^{-/-}$ mice showed an enhanced collagen-specific 'Th1-associated' IgG2a response and increased Th1-cytokine production by splenocytes as determined by an in vitro assay. Taken together, these results strongly suggest a role for the cholinergic anti-inflammatory

Extended report

Figure 6 Antigen-specific production of T helper cell 1 (Th1)-associated cytokine interferon γ (IFN γ) and the Th2-associated cytokine interleukin 10 (IL-10) by splenocytes of $\alpha 7$ subunit of nicotinic acetylcholine receptor deficient ($\alpha 7nAChR^{-/-}$) mice (n=19) and wild-type (WT) littermates (n=16), and the production of proinflammatory cytokines tumour necrosis factor α (TNF α) and IL-6. (A) The production of IFN γ by splenocytes of $\alpha 7nAChR^{-/-}$ mice was significantly higher than the production by WT splenocytes, whereas the level of IL-10 was not altered. (B) The production of TNF α and IL-6 by splenocytes of $\alpha 7nAChR^{-/-}$ mice was significantly higher than by splenocytes of WT mice. *p<0.05, **p<0.01. Mean $\pm$ SEM is shown.

pathway in the development of arthritis, which is at least in part dependent on the $\alpha 7nAChR$.

Recent data from various animal models of inflammation point towards the $\alpha 7$ subunit of the nAChR as a crucial player in cholinergic modulation of inflammation.^{7 12 36 37} Consistent with these results, we recently described for the first time the role of the cholinergic anti-inflammatory pathway in murine CIA using unilateral cervical vagotomy and treatment with an $\alpha 7nAChR$ agonist.¹⁶ In this study, we further investigated the role of cholinergic receptors in CIA using $\alpha 7nAChR^{-/-}$ mice and their WT littermates. The objective of the study was to determine the role of the endogenous cholinergic anti-inflammatory pathway, rather than the effect of exogenous stimulation of this mechanism, in the pathogenesis of CIA. Consistent with the anti-inflammatory effect of activation of $\alpha 7nAChR$ by a specific agonist, in $\alpha 7nAChR^{-/-}$ mice, we observed a marked increase in clinical arthritis scores and synovial inflammation compared with the WT littermates.

Because the effect on synovial inflammation was more pronounced in the more acute phase of the disease, we further unravelled the underlying mechanism responsible for this anti-inflammatory effect in $\alpha 7nAChR^{-/-}$ mice killed on day 44. Measuring the level of CII-specific antibody titres in the serum, we found an increase in IgG2a antibodies in $\alpha 7nAChR^{-/-}$ mice, resulting in an increased IgG2a/IgG1 ratio. This indicates that the $\alpha 7$ subunit can modulate the outcome of B-cell immunity against collagen type II. Because humoral responses induced under Th1 conditions are characterised by predominant production of IgG2a antibodies, we confirmed the switch from Th2 to Th1 profile by measuring the antigen-specific production of the Th1-associated cytokine IFN γ and the Th2-associated cytokine IL-10, produced by splenocytes after stimulation with cCII. The

level of IFN γ was significantly increased in $\alpha 7nAChR^{-/-}$ mice, whereas the level of IL-10 was not altered, resulting in a cytokine profile more skewed towards a Th1 profile.

Earlier reports have shown that stimulation or inhibition of $\alpha 7nAChR$ particularly affects TNF α production in the spleen.^{10 13 33} Since it has been hypothesised that the spleen is critical for the anti-inflammatory function of the cholinergic pathway,³³ we investigated the antigen-specific production of TNF α by splenocytes from $\alpha 7nAChR^{-/-}$ mice and WT littermates. In splenocytes of $\alpha 7nAChR^{-/-}$ mice the production of TNF α was increased. This may in part explain the observed aggravation of arthritis because as in human RA, TNF α has a pivotal role in its pathogenesis.^{38 39}

Although the experiments shown in this paper were not designed to dissect the role of individual cell populations in the inflamed synovium, they clearly support the notion that the $\alpha 7nAChR$ is intimately involved in the regulation of the inflammatory response in arthritis. Several studies have shown that the $\alpha 7nAChR$ is expressed by various immune cells, among which are monocytes, macrophages, T and B lymphocytes, dendritic cells.⁴⁰ Recent work has also shown that the $\alpha 7nAChR$ is expressed in the inflamed synovium of patients with RA as well as by fibroblast-like synoviocytes in vitro.^{20 21} It is likely that the lack of the $\alpha 7nAChR$ in the synovium as well as other sites in the body contributes to the aggravation of arthritis in $\alpha 7nAChR^{-/-}$ mice owing to non-response to endogenous ACh. An intriguing possibility is that the lack of the $\alpha 7nAChR$ in the brain is also relevant, as a recent study has shown that inhibition of brain acetylcholinesterase activity suppresses systemic inflammation through a central muscarinic receptor-mediated and vagal- and $\alpha 7nAChR$ -dependent mechanism.⁴¹ This may imply that

in $\alpha 7nAChR^{-/-}$ mice this suppression is neutralised, whereas brain development is normal in $\alpha 7nAChR^{-/-}$ mice.⁴²

Taken together, these findings show that the absence of the $\alpha 7nAChR$ is associated with aggravated arthritis in CIA. This observation supports the hypothesis that selective, peripherally acting $\alpha 7nAChR$ agonists may have an anti-inflammatory effect in arthritis.¹⁶

Acknowledgements The authors would like to thank Dr DM Gerlag for scoring the x-rays.

Competing interests GJL owns stock options of Critical Therapeutics.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

- Tracey KJ. The inflammatory reflex. *Nature* 2002;**420**:853–9.
- Ulloa L. The vagus nerve and the nicotinic anti-inflammatory pathway. *Nat Rev Drug Discov* 2005;**4**:673–84.
- van Maanen MA, Vervoordeldonk MJ, Tak PP. The cholinergic anti-inflammatory pathway: towards innovative treatment of rheumatoid arthritis. *Nat Rev Rheumatol* 2009;**5**:229–32.
- Tracey KJ. Reflex control of immunity. *Nat Rev Immunol* 2009;**9**:418–28.
- Borovikova LV, Ivanova S, Zhang M, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 2000;**405**:458–62.
- van Westerloo DJ, Giebelen IA, Florquin S, et al. The cholinergic anti-inflammatory pathway regulates the host response during septic peritonitis. *J Infect Dis* 2005;**191**:2138–48.
- van Westerloo DJ, Giebelen IA, Florquin S, et al. The vagus nerve and nicotinic receptors modulate experimental pancreatitis severity in mice. *Gastroenterology* 2006;**130**:1822–30.
- Guarini S, Altavilla D, Cainazzo MM, et al. Efferent vagal fibre stimulation blunts nuclear factor-kappaB activation and protects against hypovolemic hemorrhagic shock. *Circulation* 2003;**107**:1189–94.
- de Jonge WJ, van der Zanden EP, The FO, et al. Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway. *Nat Immunol* 2005;**6**:844–51.
- Wang H, Yu M, Ochani M, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* 2003;**421**:384–8.
- Wang H, Liao H, Ochani M, et al. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. *Nat Med* 2004;**10**:1216–21.
- Pavlov VA, Ochani M, Yang LH, et al. Selective alpha7-nicotinic acetylcholine receptor agonist GTS-21 improves survival in murine endotoxemia and severe sepsis. *Crit Care Med* 2007;**35**:1139–44.
- Huston JM, Ochani M, Rosas-Ballina M, et al. Splenectomy inactivates the cholinergic anti-inflammatory pathway during lethal endotoxemia and polymicrobial sepsis. *J Exp Med* 2006;**203**:1623–8.
- Sadis C, Teske G, Stokman G, et al. Nicotine protects kidney from renal ischemia/reperfusion injury through the cholinergic anti-inflammatory pathway. *PLoS ONE* 2007;**2**:e469.
- Giebelen IA, Le Moine A, van den Pangaart PS, et al. Deficiency of alpha7 cholinergic receptors facilitates bacterial clearance in Escherichia coli peritonitis. *J Infect Dis* 2008;**198**:750–7.
- van Maanen MA, Lebre MC, van der Poll T, et al. Stimulation of nicotinic acetylcholine receptors attenuates collagen-induced arthritis in mice. *Arthritis Rheum* 2009;**60**:114–22.
- Mullen G, Napier J, Balestra M, et al. (-)-Spiro (1-azabicyclo (2.2.2) octane-3,5'-oxazolidin-2'-one), a conformationally restricted analogue of acetylcholine, is a highly selective full agonist at the alpha 7 nicotinic acetylcholine receptor. *J Med Chem* 2000;**43**:4045–50.
- Borovikova LV, Ivanova S, Nardi D, et al. Role of vagus nerve signaling in CN1-1493-mediated suppression of acute inflammation. *Auton Neurosci* 2000;**85**:141–7.
- Pongratz G, Straub RH. The B cell, arthritis, and the sympathetic nervous system. *Brain Behav Immun* 2010;**24**:186–92.
- Waldburger JM, Boyle DL, Pavlov VA, et al. Acetylcholine regulation of synovial cytokine expression by the alpha7 nicotinic receptor. *Arthritis Rheum* 2008;**58**:3439–49.
- van Maanen MA, Stoof SP, van der Zanden EP, et al. The alpha7 nicotinic acetylcholine receptor on fibroblast-like synoviocytes and in synovial tissue from rheumatoid arthritis patients: a possible role for a key neurotransmitter in synovial inflammation. *Arthritis Rheum* 2009;**60**:1272–81.
- Wooley PH, Luthra HS, Stuart JM, et al. Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I region) linkage and antibody correlates. *J Exp Med* 1981;**154**:688–700.
- Wooley PH, Luthra HS, Griffiths MM, et al. Type II collagen-induced arthritis in mice. IV. Variations in immunogenetic regulation provide evidence for multiple arthritogenic epitopes on the collagen molecule. *J Immunol* 1985;**135**:2443–51.
- Campbell IK, Hamilton JA, Wicks IP. Collagen-induced arthritis in C57BL/6 (H-2b) mice: new insights into an important disease model of rheumatoid arthritis. *Eur J Immunol* 2000;**30**:1568–75.
- Inglis JJ, Simelyte E, McCann FE, et al. Protocol for the induction of arthritis in C57BL/6 mice. *Nat Protoc* 2008;**3**:612–18.
- Kai H, Shibuya K, Wang Y, et al. Critical role of M. tuberculosis for dendritic cell maturation to induce collagen-induced arthritis in H-2b background of C57BL/6 mice. *Immunology* 2006;**118**:233–9.
- Ort-Urtreger A, Goldner FM, Saeki M, et al. Mice deficient in the alpha7 neuronal nicotinic acetylcholine receptor lack alpha-bungarotoxin binding sites and hippocampal fast nicotinic currents. *J Neurosci* 1997;**17**:9165–71.
- Gerlag DM, Ransone LJ, Tak PP, et al. The effect of a T cell-specific NF-kappa B inhibitor on *in vitro* cytokine production and collagen-induced arthritis. *J Immunol* 2000;**165**:1652–8.
- van Holten J, Reedquist K, Sattone-Roche P, et al. Treatment with recombinant interferon-beta reduces inflammation and slows cartilage destruction in the collagen-induced arthritis model of rheumatoid arthritis. *Arthritis Res Ther* 2004;**6**:R239–49.
- Kop EN, Adriaansen J, Smeets TJ, et al. CD97 neutralisation increases resistance to collagen-induced arthritis in mice. *Arthritis Res Ther* 2006;**8**:R155.
- Svensson L, Jirholt J, Holmdahl R, et al. B cell-deficient mice do not develop type II collagen-induced arthritis (CIA). *Clin Exp Immunol* 1998;**111**:521–6.
- Delgado IM, Abad C, Martinez C, et al. Vasoactive intestinal peptide prevents experimental arthritis by downregulating both autoimmune and inflammatory components of the disease. *Nat Med* 2001;**7**:563–8.
- Rosas-Ballina M, Ochani M, Parrish WR, et al. Splenic nerve is required for cholinergic anti-inflammatory pathway control of TNF in endotoxemia. *Proc Natl Acad Sci USA* 2008;**105**:11008–13.
- Schett G. Review: immune cells and mediators of inflammatory arthritis. *Autoimmunity* 2008;**41**:224–9.
- McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. *Nat Rev Immunol* 2007;**7**:429–42.
- The FO, Boeckxstaens GE, Snoek SA, et al. Activation of the cholinergic anti-inflammatory pathway ameliorates postoperative ileus in mice. *Gastroenterology* 2007;**133**:1219–28.
- Giebelen IA, van Westerloo DJ, LaRosa GJ, et al. Local stimulation of alpha7 cholinergic receptors inhibits LPS-induced TNF-alpha release in the mouse lung. *Shock* 2007;**28**:700–3.
- Maini RN, Taylor PC. Anti-cytokine therapy for rheumatoid arthritis. *Annu Rev Med* 2000;**51**:207–29.
- Tracey D, Klareskog L, Sasso EH, et al. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol Ther* 2008;**117**:244–79.
- de Jonge WJ, Ulloa L. The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation. *Br J Pharmacol* 2007;**151**:915–29.
- Pavlov VA, Parrish WR, Rosas-Ballina M, et al. Brain acetylcholinesterase activity controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. *Brain Behav Immun* 2009;**23**:41–5.
- Yu WF, Guan ZZ, Nordberg A. Postnatal upregulation of alpha4 and alpha3 nicotinic receptor subunits in the brain of alpha7 nicotinic receptor-deficient mice. *Neuroscience* 2007;**146**:1618–28.

Role of the cholinergic nervous system in rheumatoid arthritis: aggravation of arthritis in nicotinic acetylcholine receptor $\alpha 7$ subunit gene knockout mice

Marjolein A van Maanen, Susanne P Stoof, Gregory J LaRosa, et al.

Ann Rheum Dis 2010 69: 1717-1723 originally published online May 28, 2010

doi: 10.1136/ard.2009.118554

Updated information and services can be found at:

<http://ard.bmj.com/content/69/9/1717.full.html>

These include:

References

This article cites 42 articles, 9 of which can be accessed free at:

<http://ard.bmj.com/content/69/9/1717.full.html#ref-list-1>

Article cited in:

<http://ard.bmj.com/content/69/9/1717.full.html#related-urls>

Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections

Articles on similar topics can be found in the following collections

[Degenerative joint disease](#) (2463 articles)
[Musculoskeletal syndromes](#) (2657 articles)
[Connective tissue disease](#) (2207 articles)
[Immunology \(including allergy\)](#) (2568 articles)
[Inflammation](#) (476 articles)
[Rheumatoid arthritis](#) (1670 articles)

Notes

To request permissions go to:

<http://group.bmj.com/group/rights-licensing/permissions>

To order reprints go to:

<http://journals.bmj.com/cgi/reprintform>

To subscribe to BMJ go to:

<http://group.bmj.com/subscribe/>