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EXTENDED REPORT

The anti-inflammatory effects of sympathectomy in murine antigen-induced arthritis are associated with a reduction of Th1 and Th17 responses

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ABSTRACT

Background Both facilitatory and inhibitory effects of the sympathetic nervous system (SNS) on experimental arthritis have been reported. It is unknown whether such bidirectional effects are inherent to all experimental arthritis models and/or whether critical time windows exist for influences of the SNS on inflammation.

Objectives To assess the effect of sympathectomy at different time points on the course and severity of murine antigen-induced arthritis (AIA).

Methods AIA was induced in mice. Chemical sympathectomy with 6-hydroxydopamine was carried out either neonatally, in the immunisation phase, or immediately before AIA elicitation, or during the chronic phase. In sympathectomised and non-sympathectomised AIA mice the inflammatory process (joint swelling, histopathology of inflammation and joint destruction), pain-related behaviour and cellular and humoral immune responses were analysed.

Results Sympathectomy during AIA induction or neonatal sympathectomy significantly reduced the severity of acute AIA. Neither sympathectomy in the immunisation phase nor in the chronic phase influenced AIA. Flare-up reactions were reduced by sympathectomy just before flare-up or during the initial acute AIA stage. Sympathectomised AIA mice showed less hyperalgesia. Sympathectomy significantly reduced interleukin (IL) 2, IL-17 and transforming growth factor β in supernatants from lymph nodes and/or spleen cells and antigen-specific Th1-associated IgG2a in serum; IgG1 titres were unaffected. The ß blocker, propranolol, and the norepinephrine reuptake inhibitor bupropion produced similar anti-inflammatory effects, whereas the ß-adrenergic agonist isoproterenol increased AIA severity in neonatally sympathectomised mice.

Conclusions Sympathetic activity mainly increases the severity of acute episodes of immune-mediated arthritis. Therapeutic reduction of sympathetic activity at acute stages attenuates inflammation, hyperalgesia and proinflammatory immune parameters.

INTRODUCTION

The severity of experimental arthritis can be significantly enhanced or reduced by the nervous system. Nociceptive sensory fibres support arthritis by releasing neuropeptides such as substance P and calcitonin gene-related peptide into the joint.[1] The parasympathetic system is thought to dampen inflammation.[2 3] The sympathetic nervous system (SNS) can augment or dampen arthritis.[4 5] In collagen-induced arthritis (CIA) the SNS increases inflammation in the initial phase but inhibits it at later stages.[6] Whether the SNS acts ambiguously in all forms of arthritis is unknown, and the factors which determine the direction of action are unclear. Therefore it is difficult to extrapolate firm conclusions for human disease.

In this study we explored the potential impact of the SNS on the severity and time course of murine antigen-induced arthritis (AIA), a model which shows similarities to rheumatoid arthritis (RA). In preimmunised mice the injection of the antigen, methylated bovine serum albumin (mBSA), into the knee joint causes a reproducible sequence of pathological events. Within 1 day the joint develops an acute inflammation characterised by swelling, polymorphonuclear infiltration and pronounced hyperalgesia. Within 1 week acute inflammation decreases and the joint shows a mononuclear infiltration, synovial hyperplasia, cartilage and bone destruction. A further mBSA injection evokes a flare-up reaction and aggravates destruction, similar to exacerbation in RA.[7] Here we carried out a chemical sympathectomy at different time points (neonatal, in the immunisation phase, immediately before AIA elicitation and during the chronic phase) or we applied sympatholytic or sympathomimetic compounds. In both sympathectomised and non-sympathectomised AIA mice we measured the AIA severity, AIA-induced hyperalgesia and the cellular and humoral immune responses.

METHODS

Arthritis induction

C57Bl/6 mice (7–8 weeks; Charles River, Sulzfeld, Germany) were immunised at 21 and 14 days before AIA induction with subcutaneous injection of 100 µg of mBSA (Sigma-Aldrich, Taufkirchen, Germany), emulgated with 50 µl of complete Freund's adjuvant (Sigma-Aldrich), supplemented with 2 mg/ml *Mycobacterium tuberculosis*, strain H37Ra (Difco, Detroit, Michigan, USA). Additionally, 5×10^8 heat-inactivated *Bordetella pertussis* germs (Chiron-Behring, Marburg, Germany) were applied intraperitoneally. Monarticular arthritis was induced by injection of 100 µg mBSA in 25 µl 0.9% NaCl into the right knee joint cavity (day 0 = d0). A flare-up reaction of inflammation was induced by a second intra-articular injection of 100 µg mBSA in 25 µl saline on d21 after primary arthritis induction.

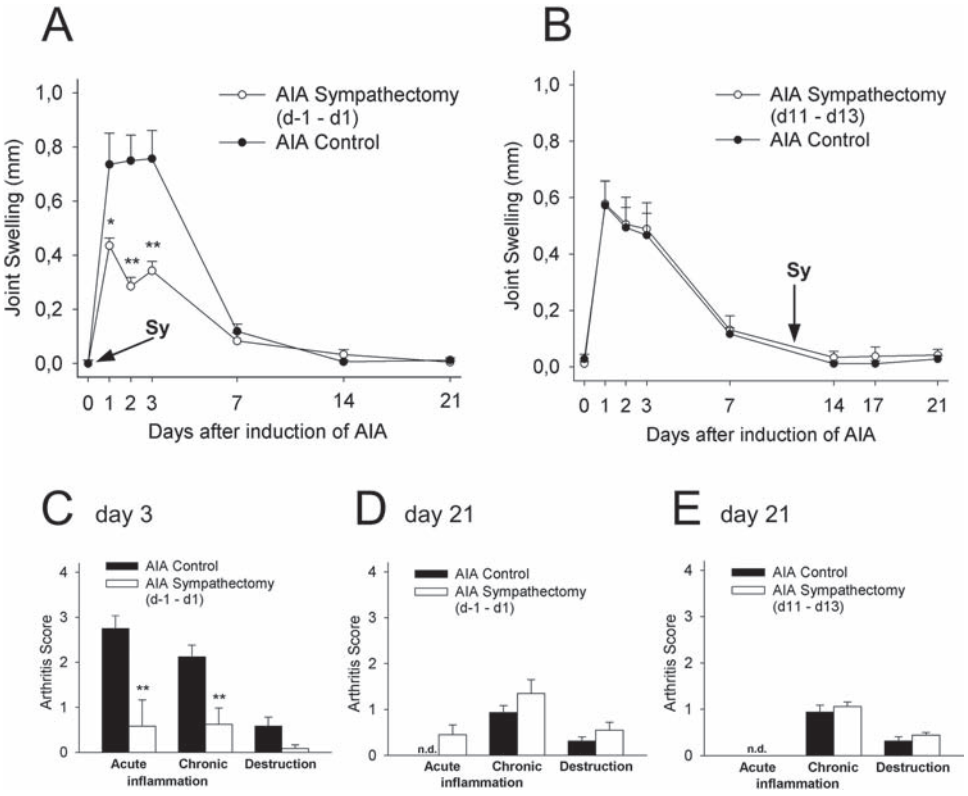

Figure 1 Sympathectomy (Sy) at the acute stage of antigen-induced arthritis (AIA) attenuates the severity of acute but not chronic AIA. (A) Sympathectomy at the time of AIA onset significantly reduces the degree of joint swelling in the acute phase of inflammation. The difference disappears at the beginning of chronic AIA (d7). (B) Sympathectomy in chronic AIA has no influence on the course of joint swelling in comparison with untreated AIA controls. (C) Mice, sympathectomised between d−1 and d1, had significantly lower scores of inflammation parameters on day 3 of AIA. (D, E) No differences in scores of inflammation parameters were seen on day 21 of AIA, neither after early sympathectomy (d−1 to d1) nor after late sympathectomy (d11 to d 13). n.d., not detectable. *p<0.05; **p<0.01 (t tests, n = 8 per group).

In 15 mice 25 µl of zymosan (12.5 mg/ml) instead of mBSA was injected into the joint. All animal studies were approved by the local government commission for animal protection.

Assessment of AIA

Swelling was assessed by measuring the mediolateral joint diameter using an Oditest vernier caliper (Kroeplin, Schlüchtern, Germany). For histopathological examination knee joints were removed, fixed in toto in 4.5% formalin, decalcified in EDTA, embedded in paraffin and cut into 3 µm frontal sections which were stained with haematoxylin and eosin. The pathologist who scored the arthritis was unaware of the animals´ treatment. Acute inflammation (infiltration of the synovial membrane by granulocytes and exudation of granulocytes into the joint space) was scored 0–3: 0 = no, 1 = mild, 2 = moderate, 3 = severe changes (+1 if fibrin exudation in the joint space)). Chronic inflammation (hyperplasia of synovial lining cells, infiltration of the synovial membrane by mononuclear cells, fibrosis of the synovial membrane and the periarticular tissue) was also scored 0–3. Cartilage surface defects with cell necrosis were scored 0–4: 0 = no damage, 1 = <5%, 2 = 5–10%, 3 = 11–50%, and 4 = >50% of the cartilage surface affected. Damage to bone was also evaluated: 0 = no, 1 = mild, 2 = medium, 3 = severe damage (extensive area of deep invasive destruction of bone).

Chemical sympathectomy and modification of adrenergic signalling

For sympathectomy in adult mice, 150 mg/kg 6-hydroxydopamine (6-OHDA; Sigma-Aldrich) in 0.1% ascorbic acid was injected on three consecutive days either during the immunisation phase, or at the time of AIA onset, or in the chronic AIA phase, or before flare-up.[6] For neonatal sympathectomy 6-OHDA was applied on five consecutive days starting 24 h after birth.[8] The ß blocker propranolol and the ß-adrenergic agonist isoproterenol (both at 10 mg/kg) and the norepinephrine reuptake inhibitor bupropion (50 mg/kg) were applied intraperitoneally at AIA onset.

Cytokine analyses

Cytokines were quantified in single-cell suspensions from lymph nodes (inguinal, popliteal, subaortic) and spleens that were removed at d3 of AIA. Cells (10^6 cells/ml) were cultured without stimulation, or with specific antigen stimulation (25 µg/ml mBSA). Cytokines were measured in the supernatants using standard sandwich ELISA procedures as previously described.[8] Primary and biotin-labelled secondary antibodies for interferon γ (IFNγ), interleukin (IL) 2, IL-4, IL-5, IL-6, IL-10 and transforming growth factor β (TGFβ) were purchased from BD Biosciences (Heidelberg, Germany), antibodies for IL-17 from R&D Systems (Wiesbaden, Germany). For quantification, recombinant cytokines were used as standard.

Serum antibody levels

Immunoglobulins (IgG) specific for mBSA and cartilage components (collagen type I and II, proteoglycans) were determined in serum, obtained at d3 of AIA, or after immunisation only, by ELISA as previously described.[9] IgG levels are illustrated as the value of absorbance representing readings obtained at 492 nm for total and cartilage-specific IgG, and at 405 nm for the IgG subclasses, respectively.

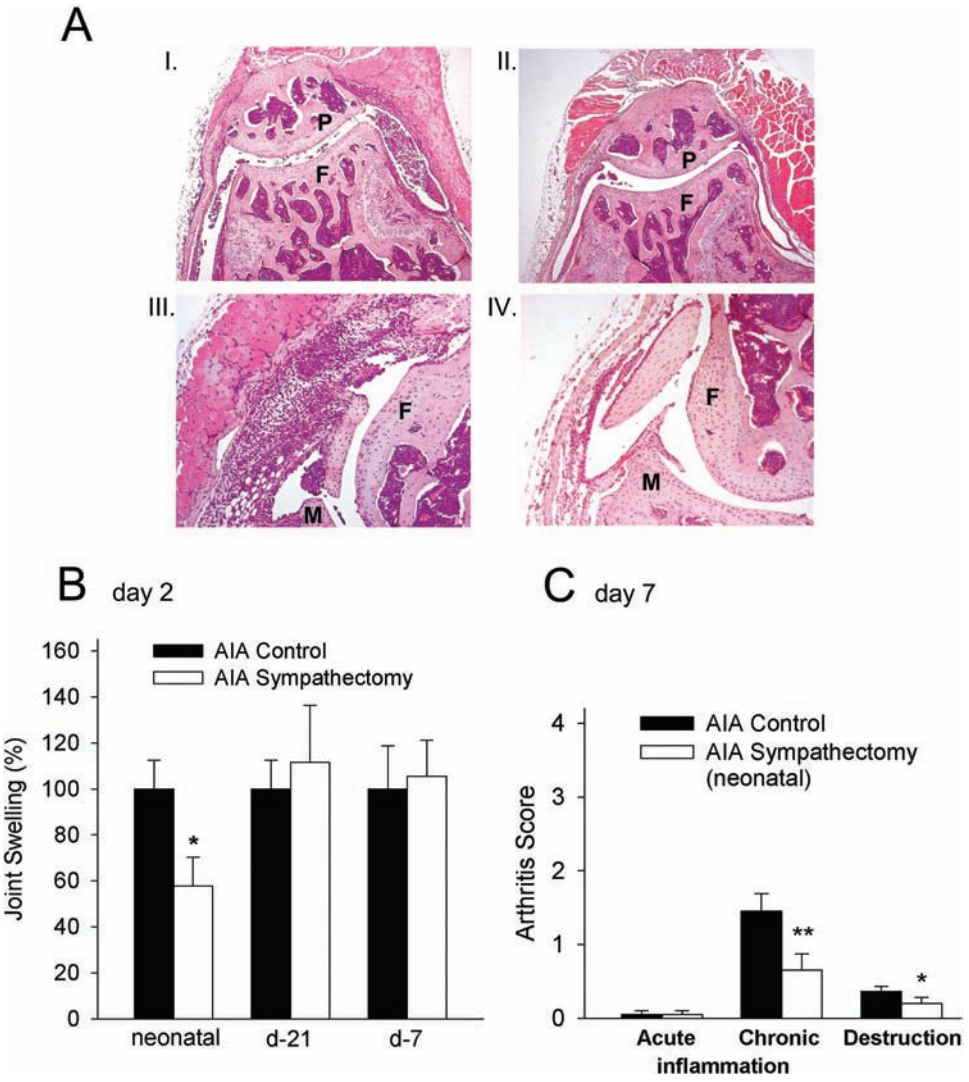

Figure 2 Flare-up reaction in antigen-induced arthritis (AIA) is affected by chemical sympathectomy (Sy). (A) Sympathectomy at the time of flare-up (d21) significantly attenuates joint swelling in comparison with untreated AIA controls. (B) Because of the prolonged duration of the flare-up reaction after the first acute inflammatory event, the histological assessment of knee joint sections was performed on day 34 (13 days after induction of the flare-up reaction), but does not show a persistent effect of sympathectomy on day 21. (C) Sympathectomy during the first induction of AIA also reduced joint swelling for 7 days in the flare-up reaction. (D) No differences in the histological assessment of joints from sympathectomised and untreated animals with AIA 7 days after induction of flare-up. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (t tests, $n = 8$ per group).

Expansion of spleen cells and white blood cells

Spleen single-cell suspensions of untreated and sympathectomised AIA mice (d3) were cultured and stimulated by mBSA for 42 h as described above. Proliferation was quantified by adding [^{3}H]thymidine (0.5 µCi/well, Amersham Biosciences, Freiburg, Germany) to the medium for the final 18 h of cell culture. In harvested cells, incorporation of [^{3}H]thymidine was measured using a microplate scintillation luminescence counter (Canberra-Packard, Schwandorf, Austria). Different white blood cells were counted by manual enumeration of May–Grünwald–Giemsa-stained blood smears.

Pain-related behaviour

Mechanical and thermal hyperalgesia at the hind paws was assessed as an indicator of secondary hyperalgesia remote from the inflamed knee joint. Mice were placed into the testing devices, and after accommodation to the environment the mechanical pain threshold was determined with a dynamic plantar aesthesiometer (Ugo Basile, Comerio, Italy) which applied increasing pressure (stimulus increase rate 1 g/s; cut-off value 10 g) to the paw. The latency of the elicited leg withdrawal, which reflects the respective mechanical threshold, was averaged from three consecutive stimuli. Two tests during the immunisation phase defined the baseline. Data are given as percentage of baseline. Thermal hyperalgesia was assessed using the Hargreaves plantar test (Ugo Basile).[10] Three consecutive standardised heat stimuli were applied to the paw for evaluation of a mean latency (cut-off value 20 s).

Statistical analysis

Data are expressed as mean ± SE. Significant differences were calculated using the two-tailed Student t test for unpaired values and the non-parametric Mann–Whitney U test by the SPSS software package (v.16.0; Chicago, Illinois, USA). Statistical significance was accepted for $p<0.05$.

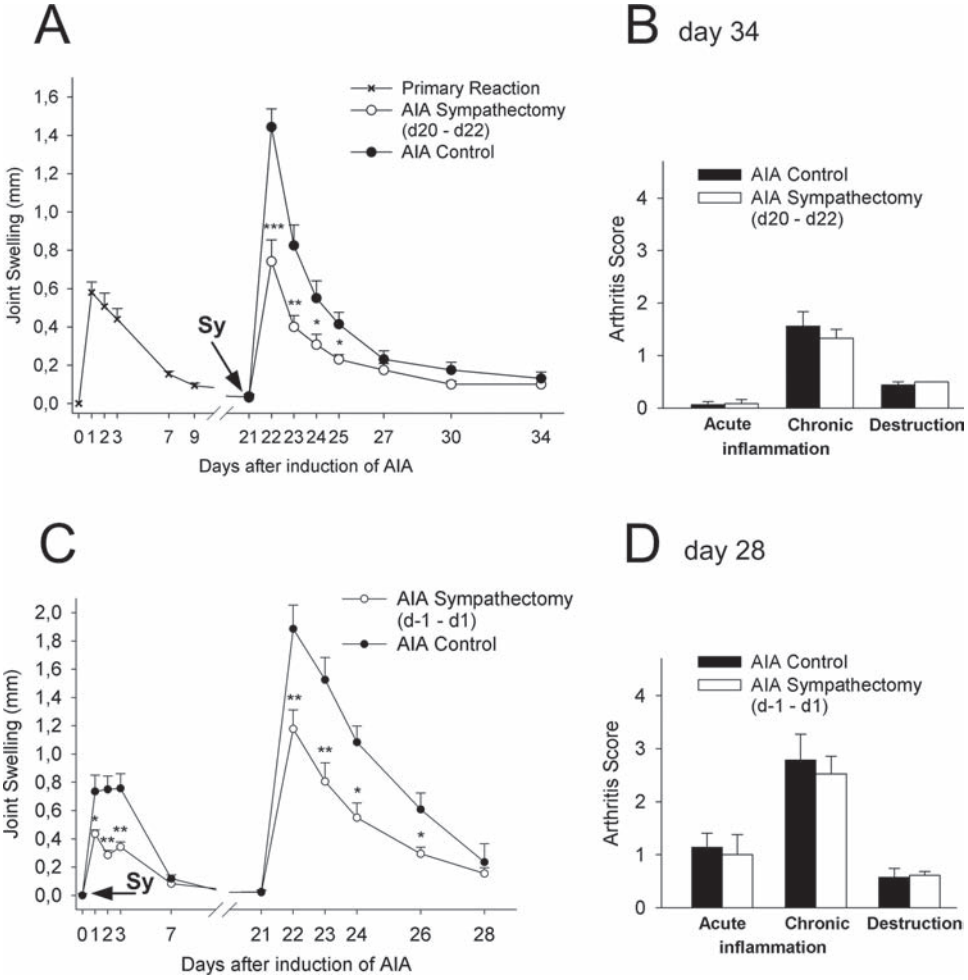

Figure 3 Typical antigen-induced arthritis (AIA) histology from sympathectomised and non-sympathectomised mice, and effects of neonatal sympathectomy and sympathectomy in the immunisation phase. (A) I and III show strong inflammation at d3 in non-sympathectomised mice, II and IV show weaker inflammation at d3 in sympathectomised mice (I and II: ×40, III and IV: ×100; F, femur; M, meniscus; P, patella). (B) Neonatal sympathectomy (t test, n = 10) significantly reduced joint swelling in acute arthritis (elicited at an age of 11 weeks) compared with untreated AIA controls. Sympathectomy within the phase of immunisation (d−21 and d−7 respectively; t test, n = 8 per group) did not affect joint swelling (values of day 2 after AIA onset). (C) Neonatal sympathectomy also significantly reduces histological arthritis score at day 7 of AIA. *$p < 0.05$; **$p < 0.01$ (U test, n = 10).

RESULTS

Effect of sympathectomy at different AIA stages on disease severity

Sympathectomy reduced norepinephrine in the spleen by 93% 1 day after 6-OHDA, and norepinephrine was restored to 83% of control level after 35 days. The number of cells in the spleen was not decreased but rather increased at d3 of AIA ($4.82 \pm 0.50 \times 10^7$ cells/ml in non-sympathectomised mice, $7.52 \pm 1.00 \times 10^7$ cells/ml after sympathectomy).

Sympathectomy did not alter the overall incidence of AIA in mice (100%). Application of 6-OHDA around AIA onset (d−1 to d1) significantly attenuated joint swelling in the acute AIA phase (figure 1A, maximum reduction of ~55% on d3) and reduced histopathological scores of acute inflammation (granulocyte infiltration and exudate) and chronic inflammation (mononuclear cell infiltration and synovial hyperplasia) at d3 of AIA (figure 1C). However, inflammation scores were not significantly different between sympathectomised and non-sympathectomised AIA mice at d21 (figure 1D). Sympathectomy at d11 to d13 of AIA neither altered joint swelling (figure 1B) nor the histopathological score (figure 1E).

Sympathectomy also reduced the severity of flare-up reactions which were induced at d21. Sympathectomy at d20 to d22 caused a significant reduction of the flare-up reaction compared with non-sympathectomised AIA mice (figure 2A). Notably, flare-up reactions were also reduced in mice which underwent sympathectomy at the primary arthritis induction—that is, before the first intra-articular mBSA injection (figure 2C). Sympathectomised and non-sympathectomised AIA animals showed similar histopathological scores of inflammation and destruction on d34 and d28, respectively (figure 2B,D), probably because the first AIA phase had already produced significant changes. Typical tissue sections of the knees from sympathectomised and non-sympathectomised mice at d3 of AIA are displayed in figure 3A.

Sympathectomy during the immunisation phase (either at d−21, or at d−7) did not influence the development of AIA (figure 3B). However, neonatal sympathectomy reduced the severity of AIA induced at an age of 11 weeks. In comparison with age-matched non-sympathectomised AIA controls, neonatally sympathectomised mice developed significantly less joint swelling (figure 3B) and arthritic signs (inflammation and destruction) until d7 (figure 3C).

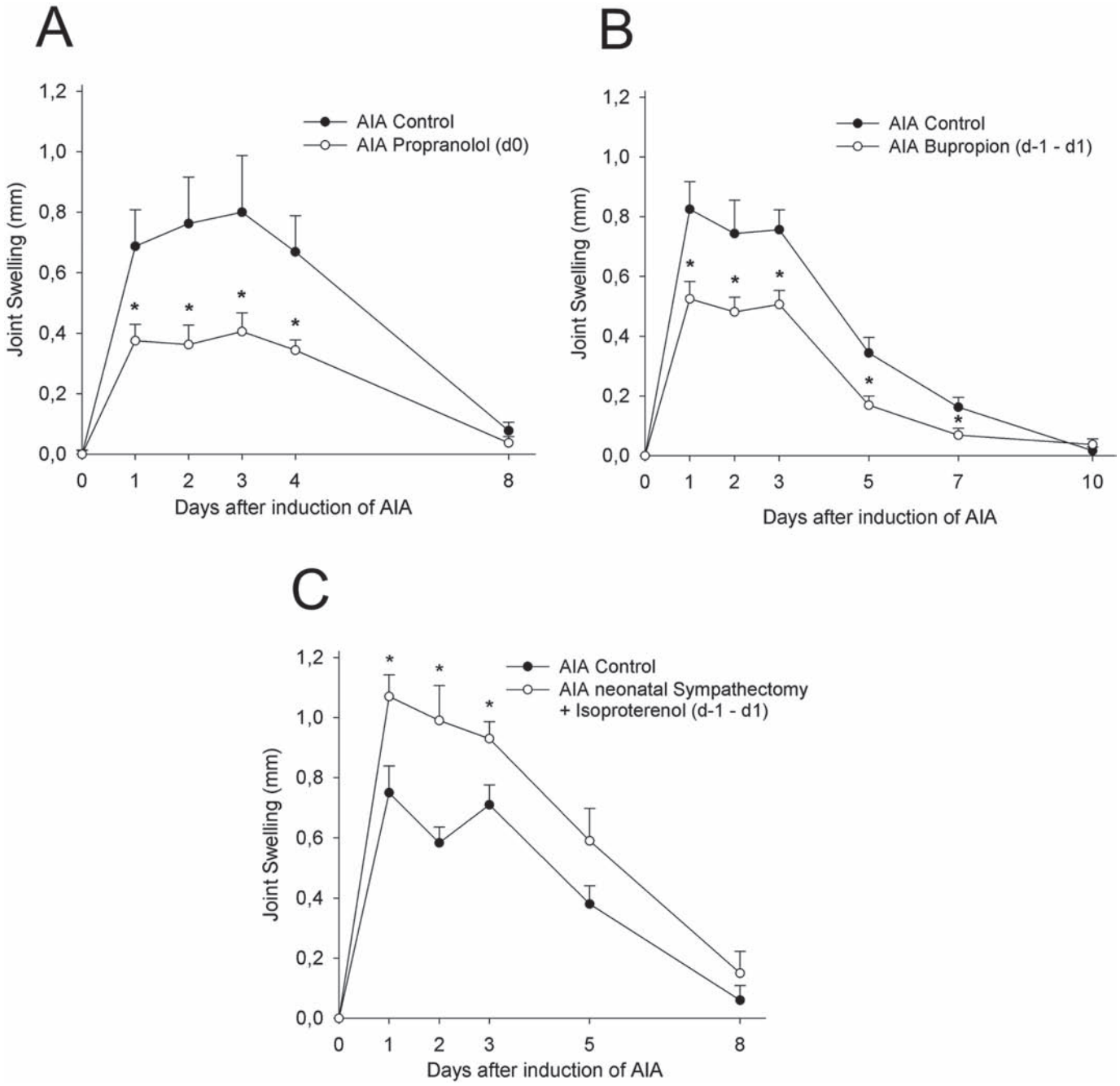

Figure 4 Sympathectomy (Sy) attenuates pain-related behaviour (secondary hyperalgesia) in antigen-induced arthritis (AIA). Mice were tested wice during the immunisation phase to obtain a baseline (BL; calculated as a mean) for withdrawal response before AIA onset. (A) Mechanical threshold (as a percentage of BL; Sy = 4.50 ± 0.15 s, control = 4.59 ± 0.15 s) for paw withdrawal in the acute AIA phase is less reduced after sympathectomy at the time of AIA onset. The difference disappears at the beginning of chronic AIA (d7). (B) Animals sympathectomised at the onset of AIA (see figure 2C), also show an elevated mechanical threshold in flare-up reaction (BL: Sy = 4.47 ± 0.14 s, control = 5.17 ± 0.13 s). (C) The time (as a percentage of BL; Sy = 4.43 ± 0.54 s, control = 4.18 ± 0.36 s) to paw withdrawal after noxious thermal stimulation is less altered in sympathectomised AIA mice than in AIA controls up to day 7 of AIA. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (t tests, n = 8 per group).

Effect of sympathectomy on pain-related behaviour during the course of AIA

Sympathectomy in non-arthritic mice did not alter response thresholds to mechanical and thermal stimuli (data not shown). AIA in non-sympathectomised mice caused a reduction of the response threshold to mechanical stimulation for paw withdrawal on the right inflamed side, indicating mechanical hyperalgesia. Sympathectomised AIA mice showed less reduction of mechanical threshold, indicating less mechanical hyperalgesia within the first 3 days of AIA (figure 4A). Mechanical hyperalgesia during the flare-up reactions was attenuated in sympathectomised mice up to d7 after AIA onset (figure 4B). The effect of sympathectomy on thermal hyperalgesia was even stronger. Thermal thresholds were significantly decreased in AIA controls but not in sympathectomised AIA mice (figure 4C).

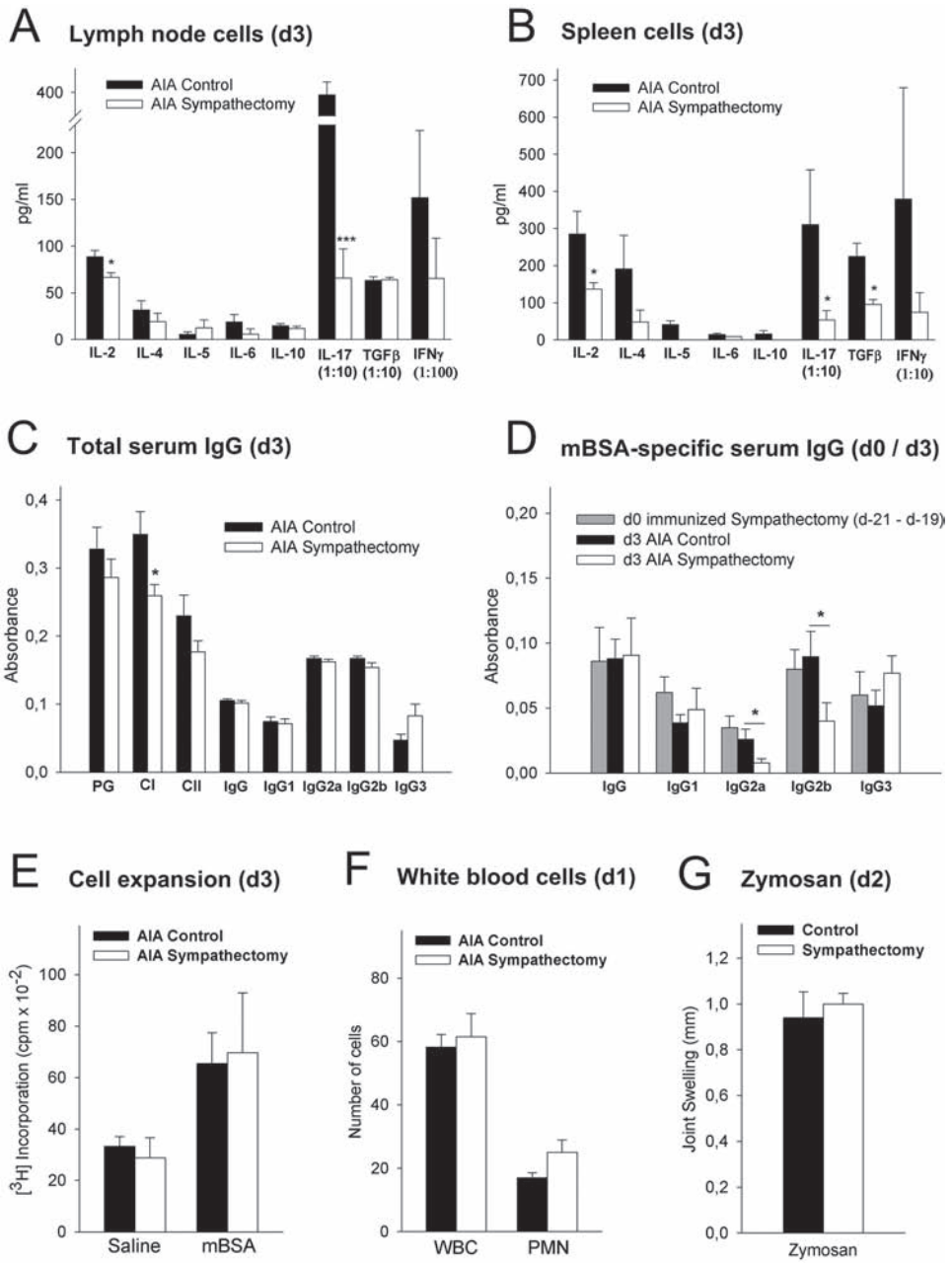

Figure 5 Cytokine expression profiles and serum immunoglobulin (IgG) levels. (A, B) Cytokine levels were quantified in supernatants of lymph node and spleen cells isolated on day 3 of antigen-induced arthritis (AIA) by ELISA after in vitro stimulation with methylated bovine serum albumin (mBSA). Supernatants of cells from sympathectomised (d−1 to d1) animals contain significantly less interleukin (IL)-2 and IL-17 than untreated AIA controls. Interferon γ (IFNγ), IL-4, IL-6, IL-10 and transforming growth factor ß (TGFß; spleen) were also diminished but this was not significant. (C, D) Immunoglobulins specific for mBSA and cartilage components (CI, collagen type I; CII, collagen type II; PG, proteoglycans) as well as total IgG were quantified in serum by ELISA. In the acute phase of AIA (d3) sympathectomised (d−1 to d1) AIA mice (white columns) had significantly less mBSA-specific IgG2a and IgG2b and CI-specific IgG than non-sympathectomised AIA mice (black columns). IgG1 and total IgG were not altered significantly. The shaded columns in D show in addition a group of mice which were sympathectomised during the immunisation phase (d−21 to d−19) but in which no AIA was induced (d0). (E) Sympathectomy (d−1 to d1) has no influence on the proliferation of spleen cells compared with untreated AIA controls neither with nor without in vitro mBSA stimulation. The proliferative response was quantified by [^{3}H]thymidine incorporation. (F) Number of total white blood cells (WBCs) and polymorphonuclear neutrophils (PMN) in blood smears of sympathectomised (d−1 to d1) and non-sympathectomised AIA mice at d1 of AIA. (G) Sympathectomy (d−1 to d1) has no influence on the joint swelling on day 2 in mBSA-immunised mice induced by injection of zymosan into the knee joint. *$p < 0.05$; ***$p < 0.001$ (t tests, $n = 6$ per group).

Effect of sympathectomy on cellular and humoral immune responses in AIA

To analyse the effect of sympathectomy on cytokine production, we quantified by ELISA the in vitro concentrations of cytokines produced in lymph node and spleen cells from either control AIA mice or from 6-OHDA-treated (d−1 to d1) AIA mice (d3 of AIA). Because there is almost no basal release of cytokines, we stimulated the cells with the recall antigen mBSA. After previous in vivo sympathectomy, IL-2 and IL-17 were significantly reduced in both lymph node and spleen cells, and in addition TGFβ was significantly reduced in supernatants from spleen cells (figure 5A,B). The pronounced reduction of IFNγ and

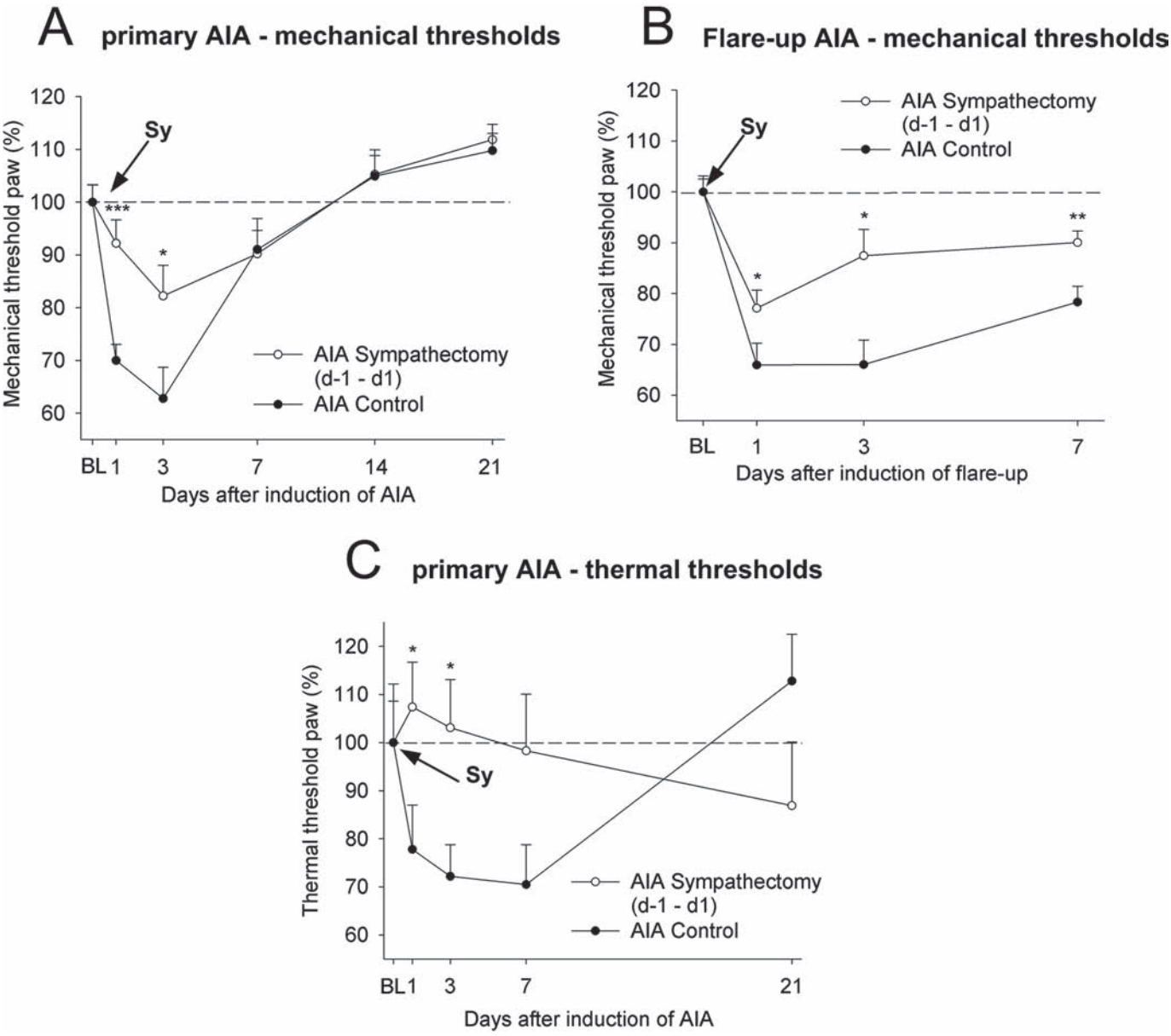

Figure 6 The severity of acute antigen-induced arthritis (AIA) is influenced by adrenergic signalling. (A, B) Application of the ß blocker propranolol (10 mg/kg intraperitoneally) and the norepinephrine reuptake inhibitor bupropion (50 mg/kg intraperitoneally) at the time of AIA onset significantly reduced the degree of joint swelling within the first days of inflammation. (C) In contrast, treatment with the ß-adrenergic agonist isoproterenol (10 mg/kg intraperitoneally) abrogates the effect of neonatal sympathectomy and exacerbates clinical parameters of acute AIA compared with untreated controls. *$p < 0.05$ (t tests, $n = 8$ per group).

IL-4 did not reach statistical significance. Sympathectomy had no influence on cell expansion. The proliferative response was similar in spleen cells from sympathectomised and non-sympathectomised mice (figure 5E).

Total immunoglobulins and immunoglobulins specific for mBSA and cartilage components were quantified by ELISA in the serum mice at d3 of AIA. Sympathectomised AIA animals had significantly less mBSA-specific IgG2a and IgG2b as well as collagen type I-specific IgG than non-sympathectomised AIA mice, whereas the IgG1 and total IgG titres were not altered (figure 5C,D). Figure 5D shows in addition that a further group of mice which were sympathectomised during the immunisation period, but in which no AIA was induced, did not show a reduction of mBSA-specific serum IgG (d0, sympathectomy d–21 to d–19 group in figure 5D). Thus mBSA-specific antibodies were not reduced by sympathectomy itself but only when AIA was induced after sympathectomy, indicating the importance of disease-promoting mechanisms.

Sympathectomy did not influence the number of white blood cells or polymorphonuclear neutrophils during AIA because the blood smears showed similar values (eg, d1, figure 5F). In order to investigate whether any inflammatory reaction itself is reduced by sympathectomy, we injected zymosan instead of mBSA into the joint. Joint swelling between 24 and 72 h after zymosan injection did not differ in sympathectomised and non-sympathectomised mice (for d2 see figure 5G).

Effects of adrenergic signalling on the severity of acute AIA

To get further evidence that the alterations in the severity of AIA are due to abrogated signalling via norepinephrine, sympatholytic or sympathomimetic compounds were applied intraperitoneally at the time of AIA onset. The ß-blocker propranolol

(figure 6A) and the norepinephrine reuptake inhibitor bupropion (figure 6B) reduced swelling similarly to sympathectomy within the acute phase of AIA compared with untreated AIA controls. Vice versa, the application of the ß-adrenergic agonist isoproterenol to neonatally sympathectomised mice (thus substituting adrenergic mediators) increased the severity of inflammation such that it was even significantly greater than AIA in non-sympathectomised mice (figure 6C). Thus these data show that pharmacological blockade of adrenergic receptors and chemical sympathectomy alter the severity of arthritis similarly, indicating a role of the SNS in inflammation.

DISCUSSION

In this study we found that the SNS significantly influences the severity of murine AIA. Chemical sympathectomy in the period of arthritis induction or at the neonatal stage significantly reduced the severity of inflammation in the acute phase of AIA but not in the subsequent chronic phase. By contrast, sympathectomy during immunisation and in the chronic phase of AIA did not influence the severity of arthritis. Flare-up reactions of AIA were reduced by sympathectomy either immediately before flare-up induction or before the first AIA induction. In sympathectomised mice both mechanical and thermal secondary hyperalgesia were attenuated in the course of AIA. We obtained evidence for a particular reduction of the antigen-specific humoral immune response and for a reduced production of IL-2 and IL-17 in sympathectomised AIA mice. As with sympathectomy, the ß blocker propranolol and the norepinephrine reuptake inhibitor bupropion attenuated acute inflammation whereas the ß-adrenergic agonist isoproterenol increased acute AIA in neonatally sympathectomised mice. These data indicate pronounced proinflammatory effects of the SNS in acute AIA.

A proinflammatory effect of the SNS was observed both in CIA and adjuvant arthritis (AA)[6 11] and now in AIA, indicating that the SNS furthers the development of arthritis across different arthritis models. As shown recently, the sympathetic activity is increased at the acute AIA stage.[12] Likewise, adrenergic antagonists attenuated AA in rats, whereas the ß-adrenergic agonist isoproterenol enhanced AA and a lipopolysaccharide-induced inflammation in mice, and similar effects were seen in this study on murine AIA.[4 13–16] However, while sympathectomy at later stages increased inflammation in CIA and AA (indicating that the SNS dampens inflammation), sympathectomy did not aggravate inflammation at any stage of AIA. The reason for this difference is unclear. In CIA the anti-inflammatory action is attributed to the appearance of non-neuronal tyrosine hydroxylase-positive cells in the joint.[17] Whether such cells are present in AIA and/or whether such cells may be killed by 6-OHDA is unknown and now under investigation.

The homogeneous and reproducible course of AIA allowed us to define which stages of inflammation are influenced by the SNS. Sympathectomy was only effective when performed during the acute stage of AIA but not when performed in the immunisation period or at the stage where signs of chronic inflammation (mononuclear cell infiltration and synovial hyperplasia) prevail (for neonatal sympathectomy see below). Notably, even when sympathectomy attenuated inflammation, the anti-inflammatory effect was confined to the acute AIA stage. At d21 control AIA mice and sympathectomised AIA mice had similar histological scores even if there is a clear difference at d3 of AIA.

Interestingly, sympathectomy at the acute stage of the first AIA episode was anti-inflammatory during the flare-up induced 21 days later. This is in contrast to the lack of effect of sympathectomy in the immunisation period. It is likely, therefore, that sympathectomy reduces inflammation only when it is coincident with an acute antigen challenge as during the acute AIA phase. Under these conditions sympathectomy may even cause an "anti-inflammatory memory" and reduce subsequent flare-up reactions. The necessity of an interaction between the SNS and the immune system is underlined by the anti-inflammatory effect of neonatal sympathectomy. Neonatal sympathectomy evokes a longlasting destruction of peripheral and central noradrenergic neurons and restrains the development of a complete immune system and, consequently, the growth and differentiation of T and B cells.[18] In adult but neonatally sympathectomised mice the acute AIA phase was less severe than in normal mice.

It is not precisely known how sympathectomy alters the severity of inflammation. Sympathetic nerve fibres of the joint control blood flow[19 20] but they may also influence immune cells in the joints.[1 2] Furthermore sympathetic fibres were shown to release prostaglandins upon stimulation,[21] and release of sympathetic neurotransmitters stimulates IFNγ secretion from T cells in early CIA.[22] Some of these local actions might promote joint inflammation. It is interesting that we found significant differences in the cellular and humoral immune responses of AIA in lymph nodes and spleen cells from non-sympathectomised and sympathectomised mice. After sympathectomy the content of IL-2, the Th1-derived IFNγ, the Th17-derived IL-17, and the possibly Treg-derived TGFβ was reduced. In particular, IL-17 has a major proinflammatory role in primary and flare-up reactions of AIA, whereas IFNγ has a pronounced pro- and anti-inflammatory role in both AIA and CIA.[9 23–26] Furthermore, sympathectomy modulated the number and activity of regulatory T cells in CIA and autoimmune encephalomyelitis.[27 28]

Of particular interest is that in AIA sympathectomy significantly decreased the amount of mBSA-specific IgG2a (Th1-associated) and IgG2b, whereas IgG1 and total IgG levels were unaffected. However, this effect was only seen when AIA was actually elicited and not when mice were just immunised and sympathectomised (see figure 5D). Thus sympathectomy mainly suppresses the disease-specific immune processes rather than the inflammatory responses themselves, and cytokines such as IL-17 may be particularly involved in this process. Collectively, these data favour the conclusion that the anti-inflammatory effect of sympathectomy results from an attenuation of the disease-specific response in the organs of the immune system rather than just from a local effect in the joint (note the lack of difference upon the intra-articular zymosan injections).

In summary, this study underlines the important role of SNS in the full expression of arthritis. Strikingly, in the AIA model the anti-inflammatory effect of sympathectomy was confined to the acute stage and did not influence the chronic pathological changes. Based on these findings, we believe that patients could benefit from a transient pharmacological reduction of sympathetic activation at acute inflammatory stages and during RA exacerbations. Such treatment is also likely to reduce the inflammatory pain because hyperalgesia is most severe in the acute stage,[29–32] and, as shown here, sympathectomised AIA mice had significantly less hyperalgesia than AIA control mice.

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The anti-inflammatory effects of sympathectomy in murine antigen-induced arthritis are associated with a reduction of Th1 and Th17 responses

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