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Catecholaminergic-to-cholinergic transition of sympathetic nerve fibers is stimulated under healthy but not under inflammatory arthritic conditions

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ABSTRACT

Objective: Density of sympathetic nerve fibers decreases in inflamed arthritic tissue tested by immunoreactivity towards tyrosine-hydroxylase (TH, catecholaminergic key enzyme). Since sympathetic nerve fibers may change phenotype from catecholaminergic to cholinergic (example: sweat glands), loss of nerve fibers may relate to undetectable TH. We aimed to investigate possible catecholaminergic-to-cholinergic transition of sympathetic nerve fibers in synovial tissue of animals with arthritis, and patients with rheumatoid arthritis (RA) and osteoarthritis (OA), and we wanted to find a possible transition factor.

Methods: Nerve fibers were detected by immunofluorescence towards TH (catecholaminergic) and vesicular acetylcholine transporter (cholinergic). Co-culture experiments with sympathetic ganglia and lymphocytes or osteoclast progenitors were designed to find stimulators of catecholaminergic-to-cholinergic transition (including gene expression profiling).

Results: In mouse joints, an increased density of cholinergic relative to catecholaminergic nerve fibers appeared towards day 35 after immunization, but most nerve fibers were located in healthy joint-adjacent skin or muscle and almost none in inflamed synovial tissue. In humans, cholinergic fibers are more prevalent in OA synovial tissue than in RA. Co-culture of sympathetic ganglia with osteoclast progenitors obtained from healthy but not from arthritic animals induced catecholaminergic-to-cholinergic transition. Osteoclast mRNA microarray data indicated that leukemia inhibitory factor (LIF) is a candidate transition factor, which was confirmed in ganglia experiments, particularly, in the presence of progesterone.

Conclusion: In humans and mice, catecholaminergic-to-cholinergic sympathetic transition happens in less inflamed tissue but not in inflamed arthritic tissue. Under healthy conditions, presence of cholinergic sympathetic nerve fibers may support the cholinergic anti-inflammatory influence recently described.

1. Introduction

In experimental arthritis and in rheumatoid arthritis (RA), sympathetic and sensory nerve fibers exert important neuronal influence (Levine et al., 1987; Bellinger et al., 2008; Pongratz and Straub, 2013). While the sensory nervous system has a proinflammatory influence, the role of the sympathetic nervous system (SNS) is bimodal with anti- and proinflammatory effects (summarized in Ref. (Bellinger et al., 2008; Pongratz and Straub, 2013)). Cholinergic anti-inflammatory effects were described in arthritis and in acute inflammation (van Maanen et al., 2009; Waldburger and Firestein, 2010; Levine et al., 2014). However, cholinergic nerve fibers are not known to innervate synovial tissue. An anti-inflammatory influence of the cholinergic pathway was described in short-standing endotoxemia (Borovikova et al., 2000). This effect is controlled by central vagal pathways (Pavlov et al., 2009). The important receptor is the nicotinergic alpha 7-subunit acetylcholine receptor (Loram et al., 2010). The cholinergic anti-inflammatory influence must happen in secondary lymphoid organs or possibly in the gut-associated lymphoid tissue but not in limbs.

The bimodal influences of the SNS depends on several factors like time of action, local nerve fiber density, and severity of

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Table 1
Characteristics of patients under study for cholinergic markers VAChT and VIP in finger joints. Data are given as means ± SEM, percentages in parentheses, and ranges in brackets.

	Osteoarthritis of finger joints	Rheumatoid arthritis of finger joints
Number	7	5
Age, yr	64.2 ± 11.4 [50–79]	63.8 ± 4.9 [59–70]
Gender male/female	2/5 (29/71)	2/3 (40/60)
Indication for surgery	7, finger joint arthrodesis	5, finger joint arthrodesis
Affected joint, proximal/distal	5/2	4/1
Affected side, right/left	6/2[a]	2/3

[a] One patient was investigated on the left and right side.

Table 2
Characteristics of patients under study for cholinergic markers VAChT and VIP in synovial tissue of the knee. Data are given as means ± SEM, percentages in parentheses, and ranges in brackets.

	Osteoarthritis of the knee	Rheumatoid arthritis of the knee
Number	44	24
Age, yr	67.7 ± 8.9 [51–86]	65.9 ± 9.4 [50–84]
Gender male/female	20/24 (45/55)	6/18 (25/75)
Indication for surgery	44, total knee arthroplasty	24, total knee arthroplasty
Affected side, right/left	23/21	13/11
C-reactive protein, mg/L	2.5 ± 2.1 [0.1–7.2]	9.5 ± 12.4 [0.5–45.0]

inflammation (summarized in Ref. (Bellinger et al., 2008; Pongratz and Straub, 2013)). In synovial membrane regions with high inflammation, sensory innervation increases and sympathetic catecholaminergic nerve fibers get lost (summarized in Ref. (Pongratz and Straub, 2013)). The loss of sympathetic nerve fibers has also been described in early inflamed pancreatic islands of BioBreeder diabetic rats (Mei et al., 2002), in the spleen of arthritic rats (Lorton et al., 2009), in Charcot foot (Koeck et al., 2009), colorectal adenomatous polyps (Graf et al., 2012), in chronic pruritus and prurigo nodularis (Haas et al., 2010), and in the colonic wall of Crohn's patients (Straub et al., 2008a).

In Crohn's disease and RA, the loss of sympathetic nerve fibers in inflamed tissue is accompanied by a relative increase of sensory substance P – positive nerve fibers. It was suggested that this phenomenon appears because of nerve fiber repulsion by semaphorin 3C/3F (Miller et al., 2004; Straub et al., 2008a; Fassold et al., 2009; Koeck et al., 2009). Sympathetic nerve fibers are immunostained by antibodies against tyrosine hydroxylase (TH, the rate-limiting enzyme of catecholamine synthesis). It might well be that this loss of nerve fibers is related to a loss of TH but sympathetic nerve fibers as such remain intact.

Most sympathetic neurons exhibit classical catecholaminergic markers such as TH. Only a minority of neurons expresses cholinergic properties (Francis and Landis, 1999). However, the cholinergic sympathetic phenotype can become dominant under certain conditions. For example, in developing sweat glands, a catecholaminergic tone is downregulated as observed by lower enzymatic activity of TH (Francis and Landis, 1999). Sympathetic nerve fibers lose catecholaminergic properties and change phenotype into a cholinergic/peptidergic type (Landis, 1996). This transition from a catecholaminergic to a cholinergic/peptidergic phenotype was also observed in the periosteum and heart muscle of heart failure patients (Asmus et al., 2000; Kanazawa et al., 2010). Such a phenomenon might also appear in inflamed synovial tissue or in tissue adjacent to arthritic inflammation (bone, muscle, skin). One may ask for the factors that induce such a catecholaminergic to cholinergic/peptidergic transition of sympathetic nerve fibers.

Glycoprotein 130 (gp130) class cytokines such as leukemia inhibitory factor (LIF), ciliary neurotrophic factor, cardiotrophin-1, and oncostatin M mimic effects of sweat gland extracts in upregulation of cholinergic markers in sympathetic neurons (Bamber et al., 1994; Geissen et al., 1998). LIF is present in synovial fluid of osteoarthritis (OA) and RA patients (Lotz et al., 1992), and LIF and other gp130 cytokines induce degradation of TH (Shi and Habecker, 2012; Nakashima, 2012). Another candidate factor is progesterone because the promotor of the vesicular acetylcholine transporter (VAChT) gene (*SLC18A3*) is equipped with a glucocorticoid/progesterone receptor binding element site (as derived using Genomatix v2.4; MatInspector Release professional 8.0.5). Under the influence of these cytokines or progesterone, sympathetic nerve fibers might lose TH and might start to express cholinergic factors such as VAChT, choline acetyl transferase, choline transporter, and vasoactive intestinal peptide (Bamber et al., 1994; Geissen et al., 1998). The subject of catecholaminergic-to-

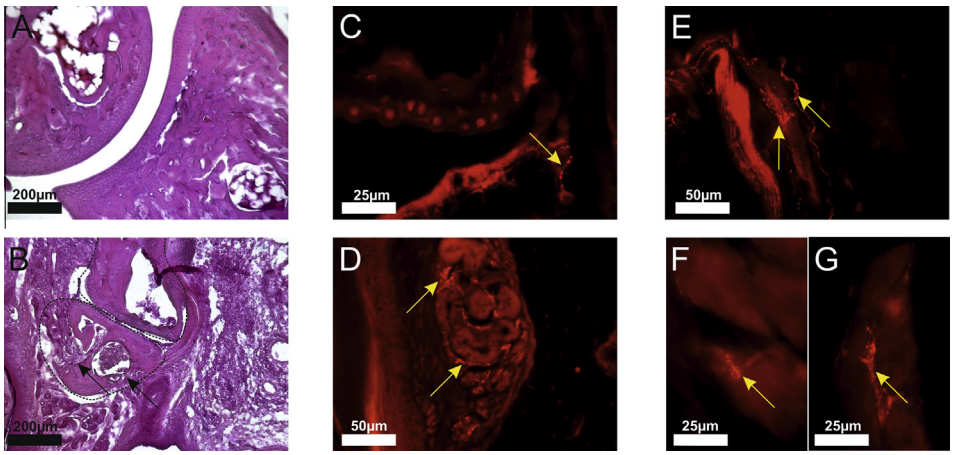

Fig. 1. Catecholaminergic and cholinergic nerve fibers in mice. (A) Histology of a normal joint in the C57Bl/6 mouse. Characteristic smooth cartilage surface at day 0 before immunization. (B) Histology of arthritic joint in the C57Bl/6 mouse at day 35 post immunization showing erosion of bone (arrows). The dotted line indicates the typical anatomy of the normal non-inflamed joint for comparison. (C–E) Catecholaminergic tyrosine hydroxylase (TH) – positive nerve fibers (arrow) in a joint (C) and surrounding blood vessels in skin of mouse limbs (D and E). (F and G) Cholinergic vesicular acetylcholine transporter (VAChT) – positive nerve fibers (arrows) in muscle (F) and skin (G) of an arthritic mouse paw.