Systematic review

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Low-grade inflammation causes gap junction-coupled cell dysfunction throughout the body, which can lead to the spread of systemic inflammation

<https://doi.org/10.1515/sjpain-2019-0061>

Received April 11, 2019; revised May 14, 2019; accepted May 21, 2019; previously published online June 28, 2019

Abstract

Background and aims: Gap junction-coupled cells form networks in different organs in the body. These networks can be affected by inflammatory stimuli and become dysregulated. Cell signaling is also changed through connexin-linked gap junctions. This alteration affects the surrounding cells and extracellular matrix in organs. These changes can cause the spread of inflammatory substances, thus affecting other network-linked cells in other organs in the body, which can give rise to systemic inflammation, which in turn can lead to pain that can turn into chronic.

Methods: This is a review based on literature search and our own research data of inflammatory stimuli that can affect different organs and particularly gap-junction-coupled cells throughout the body.

Conclusions: A remaining question is which cell type or tissue is first affected by inflammatory stimuli. Can endotoxin exposure through the air, water and body start the process and are mast cells the first target cells that have the capacity to alter the physiological status of gap junction-coupled cells, thereby causing breakdown of different barrier systems?

Implications: Is it possible to address the right cellular and biochemical parameters and restore inflammatory systems to a normal physiological level by therapeutic strategies?

Keywords: low-grade inflammation; systemic inflammation; gap junction-coupled cell networks; Ca^{2+} signaling; endotoxins; Toll-like receptors.

1 Introduction

The oral cavity and gut are complex units with a wide range of microorganisms, such as bacteria, viruses, fungi, protozoa and archaea; these microorganisms can influence these entities and by extension, influence systemic health [1, 2]. Low-grade inflammation can become chronic. Chronic inflammation can in turn lead to systemic inflammation and systemic diseases outside these units, such as cardiovascular diseases, arthritic diseases, neurodegenerative diseases, and diabetes [1, 3]. A low-grade systemic inflammation can lead to pain if the inflammation does not heal. The pain can persist and develop into chronic pain. This review focuses on endotoxin exposure in the human body, the importance of gap junction-coupled cells and how these cells are involved in inflammatory processes. Gap junction-coupled cells are found in virtually all organs in the body and react similarly in inflammatory situations *in vitro*. The question is how these cells are affected in the event of a foreign substance attack. Are other cells targeted first and alter the physiological status of gap junction-coupled cells? Moreover, can the disruption of gap junction-coupled cell networks lead to low-grade inflammation that can become chronic and systemic?

2 Methods

The review is based on literature search (PubMed) as well as our own research data of inflammatory stimuli that can affect different organs and particularly gap-junction-coupled cells throughout the body. Some clinical implications are discussed where low-grade inflammatory stimuli can develop into pain.

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3 Causes of inflammation and low-grade inflammation

Invading bacteria, viruses, and parasitic infections can cause inflammation, and the acute phase is defined as the initial pathogen invasion of any organ, which can take hours to weeks depending on the pathogen and route of infection. Mucosal immunity in the gut and oral cavity is of interest. The innate immune system is activated, and a cascade of physiological events can rapidly affect the central nervous system (CNS).

For decades, determining the initial beginning and location of an inflammatory process has been a concern in science. The oral cavity [2] and various key elements of intestinal function, such as digestion, absorption, and barrier functions, are potential initiators of inflammation [4]. However, what is the starting signal for an inflammatory process to develop into low-grade inflammation, which in the longer term can lead to chronic pain will be discussed (Fig. 1).

Fig. 1: Inflammation is a physiological response to injury that is designed to remove dangerous stimuli. Low-grade inflammation can be initiated *in vivo* after traumatic injury or in chronic diseases such as neurodegenerative, metabolic and autoimmune diseases. Gap junction-coupled network cells can be targeted, leading to the spread of inflammation and changes in biochemical cellular parameters. Astrocytes in the CNS are the most well-studied network-coupled cells that play a pivotal role in chronic neuroinflammation. This process in turn affects the surrounding cells in organs. These issues can cause the spread of inflammatory substances, thus affecting other network-linked cells in other organs in the body, which can cause systemic inflammation. Inducers of inflammation trigger the production of inflammatory mediators, which alter the functionality of tissues and organs and lead to harmful changes in different barrier systems.

3.1 The oral cavity

An interrelationship between oral bacteria and several diseases that can lead to systemic diseases has been discussed for a long time, and the teeth are the focus of infection [5]. The oral mucosa is exposed to a high diversity of microorganisms. However, the mucosa must be penetrated or damaged before antigens can modulate oral microbiome homeostasis. Nonetheless, the oral mucosa can be clinically asymptomatic but contain pathogenic bacteria. Both aerobic and anaerobic bacterial colonization exist and can act as a reservoir of inflammatory mediators such as tumor necrosis factor α (TNF- α) and interleukins, which can be released into the circulation from the diseased periodontium and be transported to distant sites of the body and cause different types of inflammatory diseases in susceptible individuals [6]. Examples of these diseases include pulmonary diseases, circulatory diseases, arthritic diseases, diabetes, and neurodegenerative diseases [3]. Moreover, proinflammatory cells and cytokines from the oral cavity can spread to the systemic circulation and increase the risk of inflammation at distant anatomical sites, such as joints and salivary glands [7]. When normal immune function becomes dysregulated during inflammation, autoantibodies can be produced [8]. For example, in rheumatoid arthritis (RA), an autoimmune response to citrullinated proteins can result in inflammation of the articular synovium and progressive destruction of cartilage and bone, which can be very painful [9, 10]. Smoking affects the oral microbiome and creates a pathogen-rich ecosystem [11]. In addition to the presence of citrullinated autoantibodies in RA, periodontitis and citrullination are strongly correlated in smokers [12, 13].

3.2 The gut

The gut microbiota is complex, and the amount of bacteria increases from the stomach through the intestines and reaches its optimum level in the colon. Genetics as well as the diet can influence the bacterial composition, thereby affecting immune cells and the immune system in the gut, which might cause low-grade inflammation [14, 15]. *Helicobacter pylori* in the stomach have been related to diseases outside the gut, and some of these diseases have been related to low-grade inflammation. Examples include cardiovascular diseases, hematological diseases, neurodegenerative diseases, respiratory diseases, ophthalmological diseases, otorhinolaryngologic diseases, and pancreatic diseases (for further information, see [1]).

Helicobacter pylori can induce problems affecting the brain-gut axis, which integrates the central, peripheral, enteric, and autonomic nervous systems, as well as the endocrine and immunological systems. Moreover, disturbances in the upper and lower digestive system tract can cause irritable bowel syndrome [16]. *Helicobacter pylori* have neurotoxic effects that can cause neurogenic low-grade inflammation. In these inflammatory processes, two cell types are potentially important: mast cells and microfold cells (M-cells) [17]. *Tropheryma whipplei* associated with human leukocyte antigen (HLA) in the intestine has been shown to disrupt the gut-joint axis and cause recurrent inflammatory processes in the joints in patients [18].

Alterations in the gut microbiota might trigger the onset of osteoarthritis (OA) through low-grade inflammation, which becomes chronic. The activity of a new metabolic OA phenotype has been suggested [15]. Overall, OA is a low-grade chronic inflammatory disease of the articular cartilage that results in pain and joint disability [19].

4 Endotoxins

Chronic exposure to low concentrations of endotoxins may give rise to symptoms originating from different organs. Low-grade inflammation can be established by subclinical doses of circulating bacterial endotoxins [20] (Fig. 2).

There is a certain risk of inhaling endotoxins, including airborne particles and aerosols. The indoor environments of water-damaged buildings contain a complex mixture of mycotoxins and different types of bacteria, such as endotoxins. Acute exposures can give rise to influenza-like reactions, such as toxic pneumonia. Chronic exposure might cause a multisystem illness referred to as “sick building syndrome”, triggering a pro-inflammatory cytokine response in the occupants. This syndrome can result in elevated leptins and decreased levels of alpha melanocyte-stimulating hormone (MSH), indicating involvement of the hypothalamus as well as deficits in neurologic function [21].

Endotoxins have been detected in 800 Swedish tap water samples, which gave rise to symptoms such as pneumonitis [22]. Endotoxin activity has also been observed in drinking water at healthcare facilities in Japan [23].

Impaired gut barrier function has been associated with increased serum concentrations of lipopolysaccharide (LPS). This issue has been observed in healthy men who showed large variations in gut permeability. Increased gut permeability was associated with elevated serum HDL-cholesterol, which was associated with serum

endotoxemia and low-grade systemic inflammation [24]. Enhanced gut permeability seems to be implicated in increasing circulating levels of LPS and a further link to increased platelet activation in patients with pneumonia complicated by cardiovascular events [25].

5 Toll-like receptors

Toll-like receptor (TLR) proteins play key roles in innate immune responses against infection. Recognition molecules bind to molecular structures in large groups of pathogens and are called pathogen-associated molecular patterns derived from invading bacteria or viruses. The TLRs belong to one of the most important pattern recognition receptor families. Currently, at least 13 different TLRs have been identified [26]. The first TLR identified was TLR4, which induces the activation and expression of NF- κ B and the generation of inflammatory cytokines [27] that are important for the inflammatory system. TLRs trigger inflammation and stimulate glial cells that induce proinflammatory mediators and cytokines [28]. TLR4 is present on astrocytes and increases its expression after LPS induction [29]. TLR4 is most likely present on all cells involved in immune function [26], including gap junction-coupled cells. Examples of these cells include chondrocytes [30], cardiac fibroblasts [31], keratinocytes [32], and tenocytes [33]. TLRs are involved in the pathogenesis of autoimmune disease, chronic inflammatory and infectious diseases, leading to overproduction of autoantibodies [34]. In OA, due to inflammation, cartilage matrix degradation leads to protein fragmentation. These fragments can act as danger-associated molecular patterns and as well as activate TLRs. Thus, persistent inflammation results in chronic activation of the innate immune response [35].

6 Gap junction-coupled cells

Gap junction-coupled cells are found in various organs throughout the body [36] (Fig. 2). These cells are typically connected through connexin-based gap junction channels. Several connexins have been discovered, but the major constituent of gap junction channels seems to be connexin 43 (Cx43), and astrocytes in the CNS are the best studied gap junction-coupled cells [37]. Gap junction channels are pore-forming and composed of two hemichannels or connexons that face each other to enable cell-to-cell communication. These channels form a ring of six protein subunits called connexins. Molecules less

Fig. 2: The role of immunological factors in the pathogenesis of chronic diseases has not been fully clarified; however, they share a systemic inflammatory response. Low-grade chronic inflammation with elevations of serum pro-inflammatory markers has been shown in patients with OA, coronary heart disease, hypertension and Alzheimer's disease. Several organs in the body comprise cells coupled into networks that communicate with each other through gap junctions. Examples of such cellular networks are astrocytes in the brain, keratinocytes in the skin and buccal membranes, chondrocytes and tenocytes in the articular cartilage and ligaments, connective tissue cells such as epithelial cells in several organs, and cardiac myofibroblasts in the heart. The body is exposed to endotoxins via inhalation, drinking water, food, and the oral cavity and microbiota in the intestine can also serve as a reservoir. Systematic inflammation affects network-coupled cells, which become inflamed and disrupted with a risk for further spread of inflammation throughout the body. The illustration was made by Pontus Andersson, ArtProduction, Gothenburg, Sweden.

than 1.5 kDa can pass through these channels. The occurrence of electrical coupling has also been demonstrated [38]. Additionally, communication between astrocytes via Ca^{2+} waves was identified [39] with a velocity of approximately 15–20 $\mu\text{m/s}$ [40]. These findings led to a proposal of syncytium-like organization. Intracellular Ca^{2+} release

is controlled by different signaling pathways that can be stimulated by different neurotransmitters, such as ATP, glutamate and 5-HT [40–42].

Hemichannels are located at the cell surface and allow the exchange of ions and signaling molecules between the cytoplasm and extracellular medium. These channels

support the uptake and release of metabolites and autocrine and paracrine communication called “gliotransmission” [43–45]. Hemichannel opening is triggered by inflammatory mediators such as the endotoxin LPS, but it does not appear to alter gap junction communication. As a consequence of hemichannel opening, enhanced glutamate release through hemichannels is observed [46]. Furthermore, the ATP concentration increases, which results in increased ATP release through hemichannels and paracrine and autocrine stimulation of purinergic receptors, resulting in increased intracellular evoked Ca^{2+} release and extracellular Ca^{2+} signaling [47].

The cells forming gap junction-coupled syncytium networks can be targets leading to the spread of inflammation and changes in biochemical cellular parameters [36]. These cells control extracellular and intracellular homeostasis at all levels of the CNS and may also contribute to the homeostasis of the other nervous systems in the body [48, 49]. The strategic organization of astrocytes from the cellular level to whole organ level plays a pivotal role in chronic neuroinflammation [50, 51].

During inflammation, the expression and affinities of several receptors, particularly inflammatory receptors such as TLR4, the substance P receptor NK-1 and the tryptase receptor PAR-2, are changed [52]. The cytoskeleton is disrupted, and Ca^{2+} signaling is elevated, resulting in increased ATP production, thereby changing the balance of Ca^{2+} -regulating processes [29, 53–55]. Increased release of ATP through Cx43 hemichannels causes Ca^{2+} propagation mediated by extracellular paracrine signaling [53]. This change in Ca^{2+} signaling causes reduced communication between cells via gap junctions [56]. Furthermore, Na^+ transporters are downregulated at the cellular level [57], increased release of proinflammatory cytokines is observed [29], and the metabolic pump Na^+/K^+ -ATPase is downregulated [58, 59].

Astrocytes are gap junction-coupled cells in the nervous system, and these cellular networks have long been proposed to lead to the spread of inflammation and changes in many cellular biochemical parameters [36, 50, 56, 60–62] (Fig. 2).

Gap junctions and Cx43 are also present in musculoskeletal tissues such as bone, cartilage, tendon and ligaments [63]. The bone cells, osteoblasts, osteocytes and osteoclasts, express Cx43, and gap junction communication seems to be important between osteoblasts for bone differentiation. Gap junction coupling has also been observed between osteoblasts and osteocytes, which might contribute to mechano-transduction [64] (Fig. 2).

The mucosa lines the epithelium and serves as a barrier that separates the lumen from the organ in most

organs in the body, including the alimentary canal, respiratory tract, genitourinary tract, and oral cavity. Epithelial cells are connected through tight junctions, adherens junctions and gap junctions, and continuous endocytosis and recycling of junctional proteins occur over the cell membrane. During inflammation, connexin degradation can cause nutrient starvation [65]. Epithelial cells in different organs express Cx43 and have gap junction communication [66], and similar properties have been demonstrated between tenocytes in Achilles tendons [67] (Fig. 2).

Chondrocytes are the main cells in cartilage, and they express Cx43 in hyaline cartilage, which form articular cartilage and the growth plate [64]. Communication between chondrocytes via Ca^{2+} waves was identified in cultured cells. Intracellular Ca^{2+} release was evoked by different signaling pathways stimulated by ATP and 5-HT. These cells also express Cx43 and TLR4 [30].

Cardiac fibroblasts in heart tissue communicate by Ca^{2+} signaling through gap junction channel Cx43 protein [31] (Fig. 2).

Keratinocytes in skin show intercellular channels, allowing intercellular exchange of small metabolites through gap junction-coupled Cx43 channels [68]. These cells as well as keratinocytes from the buccal mucosa [69] express TLR4 [32] (Fig. 2).

7 Spread of inflammation can cause systemic inflammation

Gap junction communication has been identified between different cell types in several organs. The Ca^{2+} waves in syncytium networks are dynamic signaling elements that regulate cell homeostasis in normal physiological situations. During inflammation, Ca^{2+} excitability changes.

Astrocytes are integrators and modulators in all nervous systems and control neuronal activity and synaptic transmission. A single astrocyte can make contacts with multiple neurons and capillaries and enwrap many pre- and postsynaptic terminals [70]. Therefore, astrocytes play a metabolic role in the nervous system and modulate neighboring neurons. These cells have the capacity to clear elevated K^+ from the extracellular space, take up neurotransmitters, and release gliotransmitters. Gliotransmission, a bidirectional signaling pathway, exists between astrocytes and neurons. Moreover, neural activity can trigger structural changes in astrocytes, and astrocytes can respond with long-term changes in certain properties.

Autoantibodies related to RA can develop several years before the onset of detectable joint inflammation. These autoantibodies develop outside the joints and may originate from oral, lung or gastrointestinal mucosal surfaces [71].

Metabolic disturbances can induce low-grade inflammation in all metabolically active organs, such as the liver, adipose tissue and heart, which might result in metabolic cardiomyopathy. A pro-inflammatory status and insulin resistance can account for some of these disturbances. In these conditions, glucose uptake and glucose utilization are reduced by increased fatty acid oxidization [72].

A correlation between obesity and chronic pain is a suggested link to systemic inflammation, which can develop in musculoskeletal pain, such as OA, low back pain, headaches, chronic widespread pain and fibromyalgia [73]. Adipokines such as leptin are secreted from adipose tissue and are important for the homeostasis of neuroendocrine and immune systems. Increased leptin is correlated with increased production of matrix metalloproteinases and matrix molecules, suggesting a role for leptin in the progression of OA [74–76].

Communication exists between chondrocytes and synovial cells in joints. The role and function of connexins in gap junctions in bone, muscle and joint tissue and whether communication between these different cell types occurs have been taken into consideration [64].

A remaining question is whether inflammatory reactive systemic networks in different organs possess a signaling system that can spread or propagate signals from gap junction-coupled cells in one organ to those in other organs on either the contralateral or ipsilateral side. An additional question is whether this process is an underlying mechanism of the establishment of systemic inflammation. An ongoing low-grade inflammation might give symptoms such as tiredness, widespread pain and cognitive dysfunctions [77]. Blood samples and cerebrospinal fluid give the opportunity to measure inflammation-related proteins and cytokines from patients with osteoarthritis, severe chronic pain, low-back pain and fibromyalgia [77–82].

8 Damaged barriers in the body

Inducers of inflammation trigger the production of inflammatory mediators, which alter the functionality of tissues and organs and lead to harmful changes in different barrier systems, such as the blood-brain barrier (BBB), blood-retinal barrier, blood-nerve barrier, and blood-lymph barrier [83, 84] (Fig. 1). Inflammation causes changes in

neurotransmitter systems and increased synthesis and release of pro-inflammatory mediators such as TNF- α and interleukin 1 β (IL-1 β). The affinities of several TLR receptors, especially TLR2 and TLR4, are signal sensors that recognize foreign substances. Nitric oxide synthase (iNOS) promotes increased nitric oxide (NO) production [85]. Vascular endothelial growth factor (VEGF) is considered a regulator of vascular permeability that induces leakage of the BBB by decreasing the expression of claudin-5, a tight junction protein [86].

9 Other cell types of importance

Mast cells develop in the bone marrow and circulate in the blood in low numbers as immature precursors. These cells migrate to target areas such as mucosal and connective tissues in the proximity of blood, lymphatic vessels and nerves and differentiate into mature cells in response to a currently unknown signal, such as an inflammatory signal, bacteria, virus or another agent.

Mast cells are heterogeneous with characteristic granule content [87]. These cells are the proposed first responders at the start of inflammation; mast cells respond to changes in the environment and communicate with other cells involved in the immune response, giving rise to signaling between different cell types. Mast cells have an enormous repertoire of cell surface receptors and can synthesize and release large amounts of different mediators, such as tryptases, chymases, histamine, 5-HT, nitric oxide, substance P, cytokines, chemokines, and many growth factors [87].

Mast cell progenitors can pass the BBB and establish themselves in the CNS. Once in the CNS, mast cells can interact with astrocytes, microglia and blood vessels [88]. Mast cell-released proteases activate PAR-2 receptors as well as ATP purinergic receptors expressed on both microglia and astrocytes, which can result in the release of pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-6 [52, 88]. Long-term activation of pathogenic components on gap junction-coupled astrocytes can develop into dysregulated activation that can contribute to the pathology of autoimmune diseases as well as neurodegeneration and the development of degenerative diseases [89].

Microglia in the nervous system and macrophages in other parts of the body play a pivotal role as they stimulate different substances, such as bacterial endotoxins and viruses, as well as produce and release proinflammatory cytokines, such as TNF- α , IL-1 β , IL-6 and many different growth factors. See recently published review articles [90, 91] for more details.

M-cells are located in the intestinal epithelium and localized to the luminal surface of Peyer's patches and colon lymphoid follicles [17]. These cells have contact with immune cells and seem to play an important role in the mucosal immune response by transporting external antigens from the gut lumen to the lymphoid follicle.

10 Is it possible to return the inflammatory system to a physiological level?

The role of astrocytes as dynamic modulators of synaptic functions in the neuronal environment has made astrocytes attractive targets for novel therapeutic strategies.

In our experimental systems, we have evaluated different combinations of drugs that have powerful anti-inflammatory properties. Preliminary clinical tests have shown promising results in patients [79].

One drug combination has been evaluated in our experimental system *in vitro*. This combination consists of three compounds: a μ -opioid receptor antagonist, naloxone, at ultralow concentrations; a μ -opioid receptor agonist, endomorphin-1/morphine/(-)-linalool; and the anti-epileptic agent levetiracetam. The opioid antagonist naloxone at ultralow concentrations inhibits the G_s protein of the μ -opioid receptor and activates Na^+/K^+ -ATPase activity. Opioid agonists activate the $G_{i/o}$ protein of the opioid receptor [58, 59, 92], and levetiracetam decreases IL-1 β release [93]. This combination returns the cellular parameters induced by LPS to physiological homeostatic levels and can resolve and restore disordered cellular inflammatory pathways, particularly the glutamate system.

Two of the above pharmaceutical compounds have been tested in postsurgical neuropathic pain patients with promising results [79]. We used an ultralow dose of naloxone as an adjuvant to long-term, uninterrupted intrathecal (IT) morphine infusion in patients with severe long-term inflammation and pain in whom conventional pain therapies had been insufficient. We added IT naloxone at dose levels within the nanogram level range, which were estimated to be far less than the dose needed to antagonize the effects of morphine. We found that compared to placebo, the addition of an ultralow dose of IT naloxone significantly improved the quality of sleep in these patients. Additionally, three of these 11 patients experienced pain relief [79].

As mentioned above, the increased release of ATP through Cx43 hemichannels causes Ca^{2+} propagation

mediated by extracellular paracrine signaling [94] and reduces the communication between cells with gap junctions [56]. Therefore, a search for a suitable substance that affects the ATP system in some way was desirable. The phosphodiesterase-5 (PDE-5) inhibitor sildenafil (Viagra®) was one alternative. Sildenafil induces cyclic GMP accumulation, which may inhibit inflammation [95]. Increased inflow of Ca^{2+} occurs through the N-methyl-D-aspartate (NMDA) receptor [96]. The Ca^{2+} /calmodulin complex activates iNOS, which converts L-arginine to NO, resulting in the accumulation of cyclic GMP and the activation of protein kinase G (PKG) [97]. Cyclic GMP is rapidly hydrolyzed by PDEs among which PDE-5 plays a central role [98]. PDE inhibitors exert a direct anti-inflammatory effect by raising cyclic GMP. There are indications that the NO/cyclic GMP/PKG pathway is the central signaling mechanism and can therefore be a potential tool in diseases where inflammation, including neuroinflammatory disorders, play a central role [95, 98, 99]. Sildenafil can normalize endothelial function and has been proposed for pain therapy in humans and animals [100] due to its anti-inflammatory properties. Furthermore, our group has shown that at extremely low concentrations, sildenafil works as an anti-inflammatory substance in LPS-induced inflammatory reactive astrocytes, and the number of microglia was reduced [101].

In addition, we included 1 α ,25-dihydroxyvitamin D3 (vitamin D3). Vitamin D3 acts as an immune regulator to protect against BBB disruption [102], downregulates TLR4 and decreases TNF- α and IL-6 release [103, 104]. The combination of sildenafil and vitamin D3 has positive effects on the ATP system as well as some effects on the glutamate and 5-HT systems [52].

In the future, we will refine and optimize the concentrations of the different drug combinations. For several of these drugs, only extremely low concentrations have anti-inflammatory effects. Concentrations that are too high can result in negative effects or other issues.

11 Conclusions

This review highlights gap junction-coupled cells in different organs in the body and how different sources of inflammatory stimuli can affect them. These cells form systemic networks, which might be important in low-grade inflammation that in turn can lead to systemic inflammation where pain can be a prominent symptom. These networks can be affected by inflammatory stimuli, including endotoxins such as LPS, and become dysregulated. Cell signaling decreases through connexin-coupled gap junctions but

increases through hemichannels. These changes result in decreased intercellular signaling but increased extracellular signaling that in turn affects the surrounding cells in organs. The question is which cell type or tissue is first affected by an inflammatory stimulus. Are other cells targeted first that in turn alter the physiological status of gap junction-coupled cells? One hypothesis is that mast cells mature in the organ that is first attacked by an inflammatory stimulus. Mast cells produce and release inflammatory substances, which in turn affect gap junction cell networks. The signaling through these systemic networks is disturbed, leading to dysfunctional homeostasis, which affects the cellular network's control and modulation of other target cells in the organ. The breakdown of barrier systems occurs and can cause the spread of inflammatory substances, thus affecting other network-coupled cells in other organs that can cause systemic inflammation. This, in turn, can lead to pain that can turn into chronic pain. Our own results with different combinations of pharmaceuticals together with the literature, will lead to clinical implications. Our purpose is now to continue with pre-clinical and clinical trials *in vivo*.

Acknowledgements: Thanks to Springer Nature Author Services for gold language editing.

Authors' statements

Research funding: The authors thank Edit Jacobsson's Foundation, Gothenburg, Sweden, and AFA Insurance, Stockholm, Sweden, for financial support.

Conflict of interest: Authors state no conflicts of interest.

Informed consent: Not applicable.

Ethical approval: Not applicable.

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