Review

MOLECULAR PAIN

Molecular Pain
Volume 13: 1–17

DOI: 10.1177/1744806917724559
journals.sagepub.com/home/mpx
SAGE

Immune system involvement in specific pain conditions

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Abstract

Chronic pain is a significant problem worldwide and is the most common disability in the United States. It is well known that the immune system plays a critical role in the development and maintenance of many chronic pain conditions. The involvement of the immune system can be through the release of autoantibodies, in the case of rheumatoid arthritis, or via cytokines, chemokines, and other inflammatory mediators (i.e. substance P, histamine, bradykinin, tumor necrosis factor, interleukins, and prostaglandins). Immune cells, such as T cells, B cells and their antibodies, and microglia are clearly key players in immune-related pain. The purpose of this review is to briefly discuss the immune system involvement in pain and to outline how it relates to rheumatoid arthritis, osteoarthritis, fibromyalgia, complex regional pain syndrome, multiple sclerosis, and diabetic neuropathy. The immune system plays a major role in many debilitating chronic pain conditions and we believe that animal models of disease and their treatments should be more directly focused on these interactions.

Keywords

Immune system, inflammation, chronic pain, autoimmune

Date received: 27 February 2017; revised: 20 June 2017; accepted: 7 July 2017

Overview of the immune system

The immune system is made up of two systems, the innate and adaptive immune systems, that work together to protect against infection and notify the body when/if injury occurs. The innate immune system is the in-born immune system that recognizes pathogens or injury and mounts an immediate, general response. The adaptive immune system, on the other hand, is an acquired and specific immunity. It not only responds to pathogens, but it also creates an enhanced response to future attacks based on memory. Both the innate and adaptive immune systems contain cell-mediated and humoral components. In the innate immune system, phagocytes, T cells, and cytokines form the cell-mediated response, while molecules in the extracellular fluid (e.g. complement proteins) make up the humoral response. In the adaptive immune system, cell-mediated immunity involves T cells, whereas humoral immunity involves antibodies, produced by B cells found in extracellular body fluid.

Innate immune system

The innate immune system involves ever-present cells that are prepared to recognize microbes and fight against infection on the order of minutes to hours. The main components consist of physical barriers (i.e. skin), leukocytes (macrophages and neutrophils), mast cells, natural killer (NK) cells, complement proteins, and glia.

Macrophages and neutrophils

Neutrophils and macrophages are cells responsible for the destruction of pathogens. Neutrophils reside in the bloodstream and make up the majority of the white blood cells.[1,2] In contrast, macrophages only make up a small portion of white cells,[3] but they are large and can migrate through connective tissue. They can also secrete complement proteins that together form the complement system. When one protein in the system is activated, a cascade is triggered that initiates the

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inflammatory response. Complement activation within the peripheral nerve occurs within 24 h following injury[4] and within one to two weeks within the spinal cord.[5] Both neutrophils and macrophages are phagocytic and destroy foreign pathogens by engulfing them. Neutrophil infiltration peaks within the first 24 h following injury,[6] while macrophage infiltration peaks by day 3.[7]

NK cells

NK cells, as their name suggests, are cytotoxic cells that function to kill infected cells. They do so by marking cells that need destroying and utilizing chemicals to direct a non-specific response against infected cells.[8] These substances lead to the formation of pores in the cell membrane, ultimately causing it to burst.

Mast cells

Mast cells are inflammatory cells that reside in connective tissue. Their ubiquitous nature makes them immediately able to react to pathogens. They contain granules that are expelled in response to the antibody immunoglobulin E (IgE) binding to their surface, a process called degranulation (see the study by Abraham and St John[9] for a review). This usually occurs within 24 h following injury.[7] The granules contain substances, like histamine, that lead to local swelling and redness that is associated with an injury site. Histamine functions to dilate capillaries in order to allow white blood cells to more easily pass through them.[10]

Glia

Microglia are the resident macrophages of the central nervous system. They are responsible for scavenging for damage and infectious agents in the brain and spinal cord. They are distinct from macrophages in that they are capable of changing form. In the absence of foreign or damaged material, they are in their resting, or ramified, state with their processes extended. Once activated, they take on their immune function in response to injury by retracting their processes and becoming mobile and amoeboid in shape.[11,12] Activation in the brain[13] and spinal cord[14] occurs in the first 24 h after injury and peaks in the spinal cord around one to two weeks.[15,16] They are also responsible for stimulating inflammation via extracellular signaling molecules, including cytokines. In addition, other glia, such as astrocytes, also scavenge the central nervous system (CNS). Astrocytes monitor neuronal activity and are important in repairing damaged cells and functioning as connections between capillaries and neurons. Astrocyte activation beings in the days following injury[14,17,18] and continues for at least three months.[19,20] Together, microglia and astrocytes function to recognize injury or infection, and to repair and maintain homeostasis within the CNS.[11]

Adaptive immune system

The adaptive immune system involves typically silent components that become activated in response to microbes or injury. This process follows the innate response and can take days to weeks. Humoral and cell-mediated responses are the two types of responses of the adaptive immune system and are discussed below.

Adaptive immune system: Cell-mediated response

Cell-mediated immunity involves immature T cells and their effector products that can bind antigens, much like B cells. Two major types of T cells include helper T cells and cytotoxic T cells. Helper T cells mediate the innate response by secreting chemicals that aid other cells, such as B cells. Cytotoxic T cells directly kill antigens once activated. A third type of T cell, known as regulatory, or suppressor T cells, also exist. As their name suggests, suppressor T cells are immunosuppressive. Both helper and cytotoxic T cells are further classified based on their surface proteins. The helper T cells (Th-1, Th-2, and Th-17) express a protein in the CD (cluster of differentiation) family called CD4. Therefore, they are also known as CD4+ cells, where the "+" indicates that CD4 is present on the cell surface. Similarly, cytotoxic T cells express the CD8 protein, thus making them CD8+ cells.[21] In contrast to B cells, which recognize intact antigens, T cells only recognize parts of an antigen. Therefore, in order to become activated, they must be presented with the antigen via an antigen-presenting cell (APC). Additionally, the majority of T cells only recognize major histocompatibility complex (MHC; HLA in humans) on the surface of APCs. There are two types of MHC molecules: MHCI and MHCII. MHC I molecules are located throughout the entire body on healthy cells. They present antigens from within the cytoplasm of cells and are primarily recognized by cytotoxic T cells. Therefore, CD8+ cells respond to microbes previously in the cytosol. MHC II molecules are only located on specific APCs, such as macrophages and B cells. Unlike MHC I, MHC II molecules present antigens from within the vesicles and are recognized by CD4+ helper T cells. Therefore, T cells can only bind to an antigen when it is presented to the T cell and contains the appropriate MHC molecules. In this way, cell-mediated immunity recognizes antigens that are *within* cells. Infiltration of T cells peaks around three weeks in the periphery[22] and within one to two weeks within the spinal cord.[23]

Adaptive immune system: Humoral response

B cells, and the antibodies they release, make up the humoral response in the adaptive immune system. Briefly, B cells are lymphocytes that function to recognize and bind an antigen. Depending on whether or not the antigen is T-cell dependent, T cells release additional signals and the B cell becomes activated. Once activated, the B cells differentiate and mature into plasma cells and begin to secrete antibodies. After the first exposure, memory B cells remain, so that, after a repeat exposure, the antibody response is much quicker and prolonged.[24] This is the body's way to protect against common extracellular invaders like bacteria or viruses. It is important to keep in mind that, at the first exposure, the innate immune system comes into play immediately until the B cells respond with antibody production. In response to the antibodies, the innate immune response is magnified to eliminate the antigen.

Cytokines

Secreted proteins, known as cytokines, are responsible for mediating the inflammatory reaction in both the innate and adaptive immune system. Within the innate immune system, they are released by macrophages, glia, and NK cells. In the adaptive system, T cells are the main source of cytokine secretion. Their function is to transmit signals, primarily interleukins (ILs), between cells to regulate the immune response. As their name suggests, ILs often transmit signals between leukocytes. Each cytokine has a specific target that possesses a cell-surface receptor for that cytokine. For example, macrophages secrete an activating cytokine called IL-1 that targets IL-receptors on helper T cells. Those helper T cells then secrete ILs (IL-2, IL-4, IL-5, etc.), tumor necrosis factor (TNF), and interferons (IFNγ) that activate cytotoxic and suppressor T cells by binding to their respective receptors. Cytokines are protein mediators and can be both pro- and anti-inflammatory. For example, IL-17 is pro-inflammatory[25] and is released by Th-17 cells, while IL-4 and IL-5 are anti-inflammatory and are released by Th-2 cells.[26,27] In addition, some cytokines, such as IL-6, can have both pro- and anti-inflammatory actions depending on the receptor.[28]

Substance P

Substance P (SP) is a member of the tachykinin family of neuropeptides and is believed to be important in pain transmission in the CNS, though its actions are not restricted to the CNS. It is released by peptidergic, unmyelinated C fibers following injury and primarily functions as a neurotransmitter. It binds to the neurokinin receptors (NK1 and NK2) located on immune cells, such as T cells.[29,30] The binding of SP to its receptor results in internalization of the receptor, initiates the release of cytokines[31] and stimulates macrophages.[32]

Bradykinin

Bradykinin is a peptide that mediates inflammation and plays a role in sensitizing neurons.[33] It is produced in plasma and functions to dilate blood vessels in order to lower blood pressure. It is also involved in the mechanism of pain in that activation of its receptors plays a role in the upregulation of nerve growth factor (NGF)[34] and it enhances activation of the transient receptor potential cation channel subfamily V member 1 (TRPV1).[35] Its receptors are the B_1 and B_2 receptors that belong to the class of G-protein-coupled receptors (GPCRs). While B_2 is always expressed, B_1 is upregulated after injury.[36] It has been demonstrated that blocking B_2 via a receptor antagonist can eliminate C-fiber responses, while blocking B_1 had no effect on C fiber nociception.[33] Recently, it was shown that B_1 is involved in mediating itch on inflamed skin in mice.[37]

Prostaglandins

Prostaglandins are derived from fatty acids within the cell membrane and are responsible for maintaining homeostasis and mediating inflammation. They are produced throughout the body but only act on target cells in their local surroundings. Cyclooxygenases (COX-1 and COX-2) are involved in the synthesis of prostaglandins. Generally, baseline prostaglandin levels are due to COX-1 activity, whereas COX-2 is responsible for prostaglandins following stimulation. Thus, during inflammation, COX-2 increases the prostaglandin levels. One major type of prostaglandin is prostaglandin E_2 (PGE_2). Of the ten GPCRs that recognize prostaglandins, only four specifically respond to PGE_2. These receptors are EP1, EP2, EP3, and EP4. It is now known and has been extensively reviewed that PGE_2 is involved in activation of mast cells, Th1 differentiation, and Th17 expansion.[38] Peripherally, PGE_2 is crucial in the sensitization of neurons following repetitive inflammatory stimuli by way of activation of protein kinase A (PKA) and its sodium channel Na(V)1.8 regulation.[39]

Nerve growth factor

The neurotrophic factor, NGF, is another regulator of the immune system. Following injury, within the innate immune system, it is released by mast cells in order to contribute to local inflammation.[40] Within the adaptive immune system, it is produced by the thymus and aids in T-cell maturation.

Tumor necrosis factor

TNFα is a pro-inflammatory cytokine that is produced in response to injury. The TNF family also includes TNFβ and CD40 ligand (CD40L). TNF can be produced by a multitude of immune cells, but in general TNFα is produced by macrophages and TNFβ is produced by T cells. Other cells, such as NK cells, neutrophils, and mast cells can also produce TNFα.[41] TNFα is an important mediator in both acute and chronic inflammation and its secretion leads to the production of other cytokines.

Typical immune response

When infection occurs, a cascade of events follows that result in an inflammatory reaction. The innate response usually begins when a pathogen enters the body. Pattern recognition receptors (PRRs) on glia or macrophages recognize damage or toxins and mount a general response. Neutrophils and macrophages engulf and destroy particles, while NK cells kill parasites. Other chemical defenses and the complement system come into play including mast cells, which secrete histamine and bradykinin. Pro-inflammatory cytokines, such as ILs and TNF are also released and contribute to the immune response. In concert with this innate response, the adaptive response is called to action. If an antigen is free floating, it is recognized by B cells, whereas if it has been ingested by an APC, it is presented to T cells. Following this encounter, over the course of a few days, the T and B cells become activated and begin to divide, ultimately producing their effector cells. During the following days to weeks, mature B cells (plasma cells) begin secreting antibodies, helper T cells activate macrophages, and cytotoxic T cells begin directly killing infected cells in order to eliminate the foreign material. Once the antigen has been eliminated, which could take anywhere from weeks to months, the activated B and T cells experience apoptosis and homeostasis is reestablished. While most lymphocytes undergo apoptosis, some cells remain that can more quickly divide and respond to a re-exposure, resulting in a faster and stronger immune response.

Interaction of the immune and nervous systems

A relationship exists in which the immune system can modulate the nervous system and vice versa. Now that the role of T cells within the adaptive immune response has been discussed, it is important to note that dendritic cells play a major role in T-cell activation and differentiation. Therefore, one example of this relationship would be neurotransmitter release by the nervous system regulating the interaction of dendritic cells and T cells. For instance, glutamate receptors not only were discovered to be present on human T cells, but they have also been shown to regulate T-cell function.[42] Additionally, different neurotransmitters can differentially effect the phenotypic polarization of T cells.[43]

Overview of chronic pain

Chronic pain, defined as pain lasting for more than three to six months depending on the diagnosis, arises when pain signals in the nervous system remain activated. It can result from tissue or nerve damage, inflammation, or in the absence of past insult. There is both basic science and clinical evidence that continued pain can affect the immune system and vice versa.

Peripheral pain response and sensitization

Pain transmission as a result of a noxious stimulus begins in the periphery and, by way of peripheral nerve fibers, makes its way to the central nervous system.[44] Two main types of nociceptive nerve fibers include Aδ and C fibers, though the sensory Aβ fibers also play a role. Aδ fibers are myelinated and are responsible for the initial, fast response to pain, while C fibers are unmyelinated and have a slow transmission speed. Nociceptors are typically responsible for initiating pain processing (under normal conditions) and are capable of transforming a mechanical, chemical, or thermal stimulus into an electrical stimulus.

Peripheral sensitization is an increase in peripheral afferent nerve sensitivity to stimuli which occurs after injury. This sensitivity is due to the release of inflammatory mediators, such as SP, histamine, bradykinin, ILs, TNF, prostaglandins, and NGF to name a few.[45,46] The interaction of these substances with receptors and ion channels potentiates their response and leads to a decrease in neuronal activation threshold. This drop in thresholds results in increased sensitivity to mechanical and thermal stimuli. Therefore, non-painful stimuli are perceived as painful and noxious stimuli lead to an even greater pain response. This is referred to as primary allodynia or primary hyperalgesia.[47] Additionally, previously mechanically insensitive nerve fibers are recruited and activated and there is upregulation of existing fibers adding to the nociceptive input. Peripheral sensitization is localized to the site of injury and can trigger increased excitability in the spinal cord.

Central sensitization

Activation of C fibers in the periphery, or repeated activation via exposure to an aggressive stimulus, lead to a protective (or maladaptive in the case of chronic pain) response and synaptic plasticity in the central nervous system. In this case, there is a decreased neuronal firing threshold and increased post- or pre-synaptic response.

This heightened sensitivity, termed central sensitization, was first characterized over 30 years ago.[48] Following a sensitizing event, previously subthreshold nociceptive afferents now generate action potentials in the dorsal horn. Thus, input from the periphery is important in, and can maintain, central sensitization.[49]

Another form of plasticity is windup or a temporal summation of inputs. Windup occurs when there is an increase in the firing rate of the neurons in the dorsal horn, specifically C fibers, in response to persistent low-frequency activation.[50–53] This increase is short term and occurs when stimulation is given in approximately 1 s intervals.[54] Once this repetitive stimulation is terminated, windup dissipates. Although windup is different from central sensitization, it is sufficient but not necessary for central sensitization to occur. That is, windup may lead to the development of central sensitization due to the incremental increase in firing rate, but this particular pattern of activation is not necessary to produce central sensitization.[55]

In contrast to peripheral sensitization, which typically involves an acute, local increase in sensitivity to afferent nerve stimuli, central sensitization is a longer lasting increase in response and includes input from low-threshold mechanoreceptors which are normally innocuous (allodynia), thus producing pain to normal input.[56,57] It also involves the spreading of the pain beyond the initial site of injury (secondary hyperalgesia). Thus, non-inflamed tissue around the affected area can also become sensitive and produce enhanced responses to non-painful stimuli. Once healing has occurred, if the central pathway remains excitable due to the initial damage, chronic pain can arise.

Immune cell involvement in pain

In many cases of chronic pain, there is an increase in the circulating pro-inflammatory cytokines in the blood, possibly due to an immune response, that leads to hypersensitivity. As stated previously, the innate immune system comes into action first to defend our bodies against a pathogen. This defense involves the leukocytes including mast cells, neutrophils, and macrophages. Following activation of the innate immune system, the adaptive immune system is activated and involves lymphocytes, such as T and B cells.

Peripheral sites of action

Previously it was demonstrated that mast cell depletion via compound 48/80, a mast cell degranulator, or sodium cromoglycate, a mast cell membrane stabilizer, prevented mechanical allodynia in a mouse model of postoperative pain.[58] Recently it was shown that application of azelastine hydrochloride, a mast cell stabilizer, blocked the development of mechanical allodynia and inhibited mast cell degranulation in mice with neuropathic pain.[59] These studies have demonstrated the involvement of mast cells in pain and the peripheral nervous system's contribution to chronic pain.

Macrophages, phagocytic leukocytes in the periphery, are recruited to the site of injury within three days[7] and secrete cytokines, such as ILs, which aid helper T cells and B cells as well as sensitize nociceptors. It has been demonstrated through the use of Nox2-deficient mice that macrophages contribute to neuropathic pain hypersensitivity after peripheral nerve injury.[60] Nox2 generates reactive oxygen species (ROS) production in macrophages. Thus, mice lacking the ROS-producing macrophages did not experience pain hypersensitivity suggesting their involvement in pain. Most recently, it was shown that macrophages promote the development of chronic pain in a mouse model of muscle pain. Following two acidic saline (pH 4) injections into the gastrocnemius muscle of mice, there was an increase in macrophage number and hyperalgesia. Additionally, removal of macrophages from muscle with clodronate liposomes prior to injection prevented acid-induced hyperalgesia. Furthermore, a toll-like receptor (TLR) 4 antagonist was injected intramuscularly prior to each injection. TLR4 is located on macrophages and activating these receptors increases cytokine release from the macrophages. When administered before the first injection, the antagonist attenuated hyperalgesia.[61] These studies suggest that macrophages are critical in both inflammatory and non-inflammatory pain.

Schwann cells in the peripheral nervous system are responsible for supporting neurons. Myelinating Schwann cells, as their name suggests, myelinate axons of neurons by forming the myelin sheath. Non-myelinating Schwann cells are important in maintaining the health of neurons through the production of neurotrophins (i.e. NGF). They are capable of transporting molecules across axons and can group around C fiber axons to form bundles.[62] In addition, they are also significant in the presentation of antigens to T lymphocytes. It has been shown in cultured human Schwann cells that they express both antigen-processing and -presenting machinery under basal conditions, which increases under inflammatory conditions.[63] This finding suggests that they play a role in peripheral immune responses.

Neutrophils, the most abundant white blood cell type, are involved in mediating pain. These cells are only present in injured tissue and are recruited in less than 24 h[6] to the site of injury via cytokines and chemokines (chemotactic cytokines). Neutrophil depletion has led to reduced mechanical hyperalgesia in mice following paw incision. Likewise, the treatment of mice with an antagonist for the chemokine receptor CXCR1/2, expressed on the surface of neutrophils, led to

a reduction in mechanical hyperalgesia and neutrophil infiltration.[64]

Central sites of action

There is extensive research demonstrating the role of microglia in pain.[65] They have been associated with both the initiation of peripheral pain and the maintenance of chronic pain.[66] Experiments utilizing the microglial inhibitor, minocycline, have proven effective in attenuating neuropathic pain,[67,68] and diabetic pain in rats.[69] Alternatively, when minocycline was administered in humans following lumbar discectomy to decrease perioperative leg pain intensity, persistent pain did not improve.[70] Recently, however, it was revealed that through intrathecal administration of glial inhibitors minocycline, fluorocitrate, and propentofylline, mechanical hypersensitivity is mediated through spinal microglia in male but not female mice. All three inhibitors produced a reversal in hypersensitivity in male mice following spared nerve injury (SNI) as well as in inflammatory pain.[71] Together, these results provide evidence for the importance of microglia in pain processes.

Within the adaptive immune system, T and B lymphocytes are major players. As discussed earlier, T cells are responsible for both regulating the immune response and directly killing infectious agents, while B cells are primarily responsible for secreting antibodies which recognize and eliminate pathogens.

Several animal studies provide evidence that T cells play a role in pain. One study in mice showed that administration of an antibody that led to CD4+ T-cell depletion subsequently suppressed thermal hyperalgesia and tactile allodynia following partial sciatic nerve ligation.[72] Another group examined the phenotype of T cells involved in neuropathic pain using flow cytometry. They found that neuropathic pain is mediated by Th1 cells.[73] A recent experiment demonstrated that T cells infiltrate the dorsal root ganglion following peripheral nerve injury. Once in the dorsal root ganglion, they release leukocyte elastase, a proteinase that destroys bacteria and which has been linked to chronic inflammation.[74] When leukocyte elastase was inhibited, the result was an attenuation of neuropathic mechanical allodynia in mice.[75] Costigan et al.[76] examined adult and neonatal rats following SNI. After surgery, they compared gene expression in these animals using oligonucleotide microarrays. Following analysis of the different genes, they performed immunohistochemistry, staining for Iba-1 and CD2 in the dorsal horn, and found that microglial and T-cell activation was greater in adult rats. Additionally, they used T-cell-deficient mice and measured mechanical thresholds after SNI. Mice lacking T cells had significantly less mechanical hypersensitivity compared to control animals. To further expand their findings, they also utilized mice lacking IFNγ, which is primarily expressed by T cells. Again, they found that mice lacking IFNγ showed less mechanical sensitivity after SNI. This suggests that T-cell infiltration in the dorsal horn is involved in neuropathic pain and that IFNγ plays a major role in this immune-related pain. Recently, in an investigation of sex differences in hypersensitivity, it was discovered that microglia are not required for pain in female mice following SNI. In place of microglia, females use adaptive immunity, specifically T cells, and prevention of T-cell infiltration into the spinal cord was effective in reducing pain only in female mice. Furthermore, T-cell markers CD3e, CD4, and CD8 were increased in female mice, more so than males following injury.[71] Thus, T cells clearly play a role in pain development and maintenance.

It should be noted that T cells can also play a role in pain resolution. When paclitaxel-induced mechanical allodynia was investigated in wild-type (WT) and T-cell-deficient mice ($Rag1^{-/-}$), mechanical allodynia was significantly prolonged in $Rag1^{-/-}$ mice compared to WT.[77] When $CD3^{+}$ T cells were injected into the $Rag1^{-/-}$ mice prior to paclitaxel treatment, they recovered in a comparable time to the WT mice. This suggests that T cells are necessary for chemotherapy-induced neuropathic pain recovery. Taken together, the aforementioned studies reveal that T cells play a vital role in pain processing that can differ drastically depending on the condition.

Similarly, B cells have been implicated in a number of studies to be important in pain. One group looking at interstitial cystitis (IC) performed immunohistochemistry of infiltrating B cells in addition to *in situ* hybridization. Their goal was to assess clonal B-cell expansion in tissue taken from patients with IC. Each B cell is a member of a clone, all possessing identical receptors. When this clone replicates in response to an antigen, this is known as clonal expansion. Following these experiments, they observed more B-cell infiltration and expansion in IC specimens.[78] B cells are also known to be involved with TLR signaling in that the TLR signals play a role in the removal or activation of autoreactive B cells.[79] Together, this suggests B cells can be involved in mediating aspects of pain.

Immune system receptors

Immune receptors, as the name suggests, are receptors that initiate an immune response after binding of a substance occurs. The main types include the PRRs, NK receptors (killer cell immunoglobulin-like receptors (KIRs) and killer activation receptors (KARs)), and complement receptors. Although the focus here is on how these immune receptors modulate pain, it is important to keep in mind that nociceptors can also express

these receptors. Therefore, an immune response is not always required for there to be nociception.

PRRs are part of the innate immune system and they recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). The PAMPs are microbe-specific molecules, whereas DAMPs are endogenous stress signals. The TLRs (TLR1–TLR11) are responsible for identifying PAMPs and trigger the production and release of cytokines through nuclear factor kappa B (NF-κB) signaling. Nucleotide-binding oligomerization domain-like (NOD-like) receptors (NLRs) are the main cytoplasmic PRRs and their job is to regulate inflammatory processes. They include the NODs, among other molecules. In response to molecule recognition, they activate caspases that lead to cleavage and release of cytokines and also activate NF-κB signaling. Thus, they are important in inflammatory responses to infections. In contrast, DAMPs are involved in non-infectious inflammation. For example, P2 receptors recognize nucleotides and nucleosides that serve as danger signals following damage. Specifically, $P2X_7$ recognizes excess ATP in the extracellular space and adenosine can trigger a response via the purinergic P1 receptors. Thus, both DAMPs and PAMPs are fundamental components of immunity.

Killer activation and killer cell immunoglobulin-like receptors are the receptors expressed by NK cells. They detect stress within the cell and activate or inhibit NK cell activity. When the activating signal surpasses the inhibitory signal, NK cells become activated. Cytokines, specifically IFNs, also play a role in NK cell activation. Once activated, they can bind to virus-infected cells and release proteins, such as perforin, which creates a pore in the infected cell, causing it to burst. They also secrete IFNγ and TNFα which in turn activate macrophages and promote direct killing by NK cells, respectively. These receptors have been shown to be involved in immune responses related to pain disorders, such as multiple sclerosis (MS).[80]

Complement receptors are part of the humoral response within the innate immune system and their role is to identify pathogens that are not recognized by antibodies. The four complement receptors are 1, 2, 3, and 4 (CR1, CR2, CR3, and CR4). They are part of a group of multiple small proteins known as the complement system, which aid antibodies in clearing out damaged cells and microbes. They do this via the release of cytokines that can then activate other cascades, all of which lead to the destruction or removal of foreign material. CR2 was recently shown to be increased in the spinal cord following ventral root avulsion and sciatic nerve transection in rats.[81]

Purinergic receptors are molecules found in almost all mammalian tissues. In terms of immunity, they are responsible for the secretion of cytokines. Specifically, the P2 receptors respond to ATP release. Two main classes of P2 receptors include P2X and P2Y. P2Y receptors are GPCRs that are activated by multiple nucleotides. They are located in many tissues including the brain. Microglia express many subtypes of P2Y receptors and the $P2Y_{12}$ receptor is thought to be responsible for mediating allodynia following nerve injury.[82] P2X receptors are ligand-gated ion channels that are activated by ATP. They are found among neurons and glial cells in order to regulate a variety of responses, including macrophage activation. Microglia solely express the $P2X_4$ and $P2X_7$ receptors. Their two main means of activation involve their cationic channel or their formation of a non-selective pore. It has been shown that $P2X_7$ is upregulated following injury[83] and $P2X_7$ antagonists can alleviate allodynia.[84] $P2X_7$ has also been implicated in pro-inflammatory cytokine release.[85] Further, activation of $P2X_7$ by ATP activates the NOD-like receptor pyrin domain 3 (NLRP3) in order to cleave pro-IL-1β to its mature form.[86] Following lipopolysaccharide (LPS) exposure, it was shown that P2X mediates the release of TNFα, IL-1β, and nitric oxide in macrophages.[87] Moreover, it has been demonstrated that $P2X_7$ pore formation is responsible for mediating allodynia and pain intensity in mice and humans, respectively. Variability seen in experimental pain in mice strains and chronic pain in humans was found to be due to genetic differences in $P2X_7$ receptor function.[84]

Similar to $P2X_7$, $P2X_4$ receptors have been shown to play a role in microglia signaling after injury. For instance, following injury, chemokine release from neurons leads to an increase in $P2X_4$ expression which results in the synthesis and release of brain-derived neurotrophic factor (BDNF). This leads to the downregulation of the K^+ CL^- cotransporter, KCC2, through the tyrosine kinase B receptor. This downregulation results in a disinhibition of gamma-aminobutyric acid receptors. This results in an alteration of network excitability.[88] Additionally, following spinal nerve injury, $P2X_4$ receptors were induced in activated microglia, demonstrated via immunofluorescence, and their expression was significantly increased in the spinal cord on the injured side. When TNP-ATP, an antagonist of the $P2X_{1-4}$ subtypes, was administered, withdrawal thresholds increased in a dose-dependent manner. To further elucidate which subtype was responsible, an antagonist of the $P2X_1$, $P2X_2$, $P2X_3$, $P2X_5$, and $P2X_7$ subtypes was administered and had no effect on withdrawal thresholds. Together, these data strongly suggest that $P2X_4$ receptors in microglia were responsible for the allodynia after nerve injury.[89] To take these findings a step further, mice lacking $P2X_4$ were examined following acute pain stimuli, administration of CFA (complete Freund's adjuvant) and after nerve injury. The results revealed that $P2X_4$ was not involved in all pain responses, but it

played a major role in inflammatory and neuropathic pain. Mice lacking $P2X_4$ receptors had a reduction in CFA-induced inflammatory pain and allodynia due to nerve injury.[90] Finally, these findings were confirmed and the mechanism was discovered by Trang et al.[91]. In their study using primary microglia cultures, P2X4 receptors were stimulated with ATP. In response to stimulation, they released BDNF in a Ca2+-dependent manner, which was mediated by p-38 mitogen-activated protein kinase (MAPK) activation. P38-MAPK is a kinase that has been implicated in hypersensitivity due to peripheral nerve injury.[15]

Immune system involvement in chronic conditions

There is a large number of chronic pain conditions that are classified by type or site of injury. However, there are certain conditions in which there is no evident cause for pain. Regardless, the immune system has been implicated in a number of these chronic conditions. The remainder of this review is focused on these types of conditions and the relationship between the immune system and the pain experienced.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disease which primarily affects the joints.[92] The primary characteristics are swollen, tender, and painful joints, but other parts of the body may be involved as well. RA is considered an autoimmune disease due to the presence of multiple autoantibodies, including rheumatoid factor and anti-citrullinated protein antibody (ACPA).[93,94] These antibodies can be present long before an individual is diagnosed.[95]

Although the exact cause remains to be elucidated, it is believed that both genetic and environmental factors play a role in the etiology of RA. In brief, it is thought that CD4+ T cells recognize antigens in synovial (joint capsule) tissue, following non-specific inflammation, and then stimulate other cells (i.e. macrophages) to release cytokines responsible for eroding cartilage and bone.[96] It has been suggested that circulating ACPAs target osteoclast precursors which leads to increased amounts of IL-8 and results in the initial step of RA pain.[97] An interesting aspect of RA is that pain may be present prior to inflammation of the joint and following anti-inflammatory treatment. Recently, this phenomenon was investigated by Wigerblad et al.[98] Using antibodies from humans with and without RA and murinised monoclonal ACPA, it was demonstrated that antibody injection led to prolonged nociceptive behavior in mice. This pain behavior was seen in the absence of inflammation. The mouse osteoclasts were also stimulated with antibodies and the behavioral effect was linked to osteoclast activation and subsequent release of CXCL1 (IL-8 in humans). To further confirm the involvement of ACPA, the CXCR1/2 antagonist reparixin was administered. Following treatment, antibody-induced pain behavior was reversed in mice.[98] These findings suggest that CXCL1/IL-8 release is responsible for producing pain experienced in RA.

TNFα has also been shown to play a major role in RA. It has also been suggested that synovial inflammation alone is not sufficient to explain the pain reported in RA and the animal models used to study this condition do not take into account the involvement of the immune system. Therefore, a K/BxN serum transfer model has been investigated as a model of arthritis pain. In this model, K/BxN mice produce autoantibodies against a protein present in joints and subsequently develop inflammatory arthritis. Serum from these mice is then transferred to other strains to induce temporary inflammatory arthritis. Using this model, animals were tested for hypersensitivity at the peak inflammation phase and post-inflammation phase. During the peak of acute inflammation, TNF and COX inhibitors, etanercept, and ketorolac, respectively, were administered.[99] Inhibition of the inflammatory mediators led to an attenuation of allodynia, suggesting involvement of TNF and prostaglandin in RA pain. Based on the information provided by these studies, common RA treatments involve TNFα and IL-6 inhibitors, as well as immunosuppressants.

Osteoarthritis

Osteoarthritis (OA) is the most prevalent chronic, degenerative joint disease. Although OA can affect any joint, the most commonly affected areas include the hand, neck, knee, and hip joints, as well as the lower back. Unlike RA, OA affects only the joints and involves the breakdown of cartilage and synovial inflammation.[100] Together, these cause symptoms of joint pain and stiffness.

It has been known for some time now that OA involves chronic inflammation.[101] This is evidenced by the presence of T cells, B cells, and infiltrating macrophages in the synovial membrane in multiples cases.[102] There is also evidence of involvement of the complement system and synovial macrophages in the development of OA. For example, one group performed immunohistochemistry and an enzyme-linked immunosorbent assay (ELISA) to measure terminal complement complex (TCC) deposits in synovial tissue of OA patients. They found that TCC levels were present in inflamed synovial tissue and those levels correlated with the amount of inflammation.[103] It has also been shown that complement proteins are upregulated in synovial tissue during

a flare up.[104] Despite the large amount of evidence implicating the importance of the innate immune system in its initiation,[105] it was not until recently that there was direct *in vivo* evidence that activated macrophages are involved in human knee OA. The imaging technique FolateScan was utilized as a way to detect activated macrophages via the binding of folate receptor β to macrophage surfaces. They found activated macrophages in the majority of patients and both synovial and joint capsular macrophages were associated with osteophyte severity and knee symptoms.[106] Thus, in recent years, the role of the immune system has been explored in hopes of finding potential therapeutics to treat the symptoms and progression of this disease.

According to one survey, arthritis pain was one of the most common causes of chronic pain reported in patients.[107] Both human and animal studies have been performed to reveal the link between immune system activity and the pain that accompanies OA. One such study utilized a surgical mouse model of knee OA to mimic its long-term progression. They found that, after surgery, WT mice experienced macrophage infiltration in the dorsal root ganglia, while mice lacking the chemokine (C-C motif) receptor 2 (CCR2) did not experience infiltration. Of note, animals lacking CCR2 did not exhibit pain behaviors induced by movement despite structural joint damage and allodynia comparable to WT mice. This finding suggests that CCR2 is fundamental to the development of pain with knee OA.[108]

To discover the relationship between OA pain and inflammation, primary cultures from human or newborn mouse chondrocytes were stimulated with IL-1β or its product visfatin/nicotinamide phosphoribosyltransferase. This stimulation led to an increase in NGF.[109] It has been known for some time that NGF is involved in OA pain.[110] Therefore, the authors suggest that the overexpression of IL-1β and its product may mediate OA pain by stimulating NGF release. Recently, a study involving a rat model of OA showed that a single injection of anti-NGF antibody resulted in significant reduction in pain;[111] thus, making this suggestion plausible.

A very recent study was able to build upon these findings with human patients diagnosed with knee OA. In this eloquent study, the immune cell composition of the synovium and infrapatellar fat pad, two areas highly affected in OA, was compared. Using flow cytometry, the authors were able to characterize these areas and found that they were similar in composition and that activated macrophages and T cells were abundant in these tissues, along with mast cells. Importantly, the number of helper T cells present in the synovium was associated with Visual Analog Scale pain scores.[112] Not only do these data suggest an overall inflammatory state, they also indicate that CD4+ T cells could play a role in pain sensation in patients with knee OA.

Fibromyalgia

Fibromyalgia (FM) is a chronic pain syndrome that involves widespread musculoskeletal pain.[113] Patients also complain of fatigue, memory loss, insomnia, and depression.[114,115] It is thought that patients with this syndrome have a problem with pain processing and not simply a pain experience that is localized to a certain part of the body.[116,117] In a longitudinal study of patients with FM, IL-8 in serum was elevated in FM patients[118] suggesting that FM is related to higher levels of pro-inflammatory cytokines. Recently, one group set out to compare serum concentrations of several cytokines in patients with FM. Their results revealed increased levels of IL-6, IL-10, and IL-1β in FM patients.[119] There is also evidence showing the involvement of chemokines in this condition. Serum concentrations of chemokines in FM patients were determined using ELISA. It was demonstrated that FM patients had higher levels of the inflammatory chemokines TARC/CCL17, MIG/CXCL9, MDC/CCL22, I-TAC/CXCL11, and eotaxin/CCL11.[120] Taken together, the increased levels of cytokines and chemokines suggest that there is a constant state of systemic inflammation in patients that could be related to the pathology of FM.

Recently, the relationship of leptin concentrations in pain was investigated. Leptin is a known adipokine[121] that is directly involved in activating the immune system and can lead to pro-inflammatory cytokine release.[122] The authors obtained blood samples from women experiencing pain, some of which were diagnosed with FM. Leptin levels were associated with self-reported pain and higher levels were indicative of more pain.[123] The increased levels of leptin could be responsible for the increased levels of cytokines that are also seen in FM.

FM is considered as the most common cause of generalized pain in women of middle age.[114] Diagnosis and treatment remain inadequate, necessitating the investigation of the source of pain. Many studies aimed at this question have investigated the role of the immune system in the development of FM pain. One group used blood mononuclear cells from FM patients along with animal models to determine the role of the NLRP3 inflammasome. They found increased levels of IL-1β that were positively correlated with pain scale scores in both mice and patients.[124] This was the first study to show that inflammasome activity is associated with increased pro-inflammatory cytokines in patients with FM and suggests a direct link between inflammation and pain. In line with this idea, anti-inflammatory treatments have been investigated in this condition but FM does

not seem to respond to traditional anti-inflammatory medication. However, a novel treatment involving low-dose naltrexone (LDN) has been shown to be effective. LDN is a competitive antagonist of mu-opioid receptors and has been shown to inhibit cytokine expression,[125] perhaps through glial cell inhibition. Additionally, naloxone (a chemically similar antagonist) has been shown to exhibit suppressing effects on microglia.[126] Using neuron-glia co-cultures pretreated with naloxone and subsequently treated with LPS, it was demonstrated that naloxone blocked LPS-induced inflammation and microglial activity.[126] Similarly, naltrexone was investigated in mice following LPS injection. It was found that administration of naltrexone inhibited TNFα production.[127] Due to the favorable preclinical data, a clinical trial was carried out in which patients received placebo for two weeks followed by LDN for eight weeks. Treatment with LDN reduced FM symptoms in patients and is thus a promising novel therapeutic treatment for FM patients.[128] Although the central nervous system has traditionally been seen as playing a major role in FM, there is evidence that a significant number of patients also have peripheral neuropathy. Multiple studies have demonstrated a decrease in epidermal nerve fiber density in FM patients.[129,130] Additionally, a variety of techniques has been utilized and further demonstrated peripheral abnormalities in FM.[131,132] Of note, all of these studies report small fiber neuropathy (SFN) in FM patients. Although SFN symptomology differs from FM in that it involves distal pain, medication used to treat SFN can alleviate pain associated with FM. Therefore, a recent study aimed to investigate ultrastructural changes in both FM and SFN. They found that patients with FM but not SFN had decreased axon diameters suggesting different pathology. Thus, they consider that the decreased diameter may come before small fiber loss.[133] The root of this reduction remains unknown. However, in light of the immune involvement seen in FM as well as in SFN,[129] it is plausible that the immune system plays a role in SFN as a peripheral element in FM.

Complex regional pain syndrome

Complex regional pain syndrome (CRPS) is a chronic, debilitating, and painful condition that is characterized by severe and continuous pain.[134] The cause remains unknown; however, it involves inflammation and sensitization.[135] The role of inflammation in CRPS treatment is a growing field.[136–138]

Several experiments using tibia fracture and casting animal models have been performed looking at aspects of the immune system in CRPS pain. In this model, a closed fracture of the tibia is made using a hemostat and the hindlimb is subsequently wrapped in casting tape to fix the hip, knee, and ankle. After three weeks, the cast is removed. Using this model, one group revealed that mice with depleted levels of B cells, or completely lacking them, experienced a reversal of nociceptive sensitization. Additionally, some mice failed altogether to fully develop CRPS after fracture and casting.[139] These results highlight the importance of B cells in the development and progression of CRPS.

Due to previous evidence implicating immune activation in CRPS,[140] it was suggested that modulating the immune system could prove to be an effective treatment for CRPS.[141] To do this, intravenous immunoglobulin (IVIG) was used in a clinical trial to assess its effectiveness as a therapy. IVIG involves IgG from multiple donors that is meant to modulate complement activation, suppress antibodies, block macrophage Fc receptors (receptors which bind to antibodies), and suppress inflammatory mediators.[142] In this way, the authors were able to reduce participant's pain intensity and were the first to show that immune intervention is an effective treatment for CRPS.[141,143]

The role of TNFα in CRPS pain has also been explored in both humans and animal models and there seems to be mixed results in terms of its involvement. An experiment looking at mechanical hyperalgesia in humans used measurements of soluble TNF receptor type 1 (sTNF-RI) to investigate the role for TNAα in CRPS. The level of sTNF-RI predicted the presence of mechanical hyperalgesia, in that patients without hyperalgesia had lower levels compared to patients with hyperalgesia. This suggests the involvement of TNFα in CRPS-related mechanical hyperalgesia.[144] Similarly, a rat model was used to investigate the significance of TNF signaling in mediating the chronic nociceptive sensitization by utilizing the same sTNF-RI treatment. The treatment reversed the mechanical allodynia after fracture indicating TNF is significant to the development of pain in CRPS.[145] Additionally, nociceptive sensitization was examined in a rat model of CRPS. Following tibia fracture, there was an increase in TNFα, IL-1β and IL-6 mRNA, and protein levels. Treatment with the cytokine inhibitor pentoxifylline reduced the mRNA expression and protein levels of these cytokines, displaying the contribution of pro-inflammatory cytokines in CRPS-related pain and sensitivity.[146] On the other hand, levels of IL-1β, IL-6, and TNFα in the cerebrospinal fluid of CRPS patients were compared to levels found in other patients with or without painful conditions. Employing an ELISA revealed significant increases in IL-1β and IL-6 but not TNFα in patients with CRPS.[147] This may be due to the fact that TNFα is increased locally but not systemically in CRPS patients.[148] Thus, TNFα may be involved in CRPS pain, but more research is needed to confirm its exact role in patients. Together, these findings illustrate the

clear connection between immune system involvement and pain experienced in CRPS.

Multiple sclerosis

MS is a disease in which the nerve cells of the central nervous system become demyelinated and damaged. This leads to physical and neurological problems including loss of vision, muscle weakness, loss of coordination, and cognitive impairment.[149] It is believed to be either an autoimmune disease or due to oligodendrogliopathy.[150] The most commonly accepted hypothesis is that autoimmune inflammation leads to demyelination through the activation of T cells which cross the blood brain barrier and induce the demyelination via release of cytokines and other factors.[151,152] Pain is a common symptom in MS and may be of nociceptive or neuropathic in origin,[153,154] although central neuropathic pain is most often reported.[155]

Experimental autoimmune encephalomyelitis (EAE) is the common animal model for MS. Induction involves either subcutaneous injection of peptides normally present in the CNS emulsified in adjuvant or by the transfer of T cells from mice already sensitized. It is a T-cell-dependent disease that involves infiltration of leukocytes from the blood into the CNS, a key component to the development of MS.[156] This model mimics the demyelination seen in MS. Using this model, a CX3CR1 inhibitor was used to test its efficacy in MS treatment. CX3CR1 is a chemokine receptor that binds CX3CL1 (fractalkine, FKN). Chemokines are released at sites of inflammation and are involved in mediating cell migration to inflammatory sites. CX3CR1 is specifically expressed by monocytes, T cells, and NK cells. Following administration of the inhibitor via osmotic mini pumps, EAE development was blocked and activated microglia and macrophages were reduced in the spinal cord.[157] This supports the idea that inflammatory processes mediated by chemokines are crucial to the development of MS.

Recently, it was demonstrated that pain induction can stimulate relapse in MS. Using a mouse model of EAE, the trigeminal nerve was ligated in EAE recovered animals. In addition, capsaicin, the active component in chili peppers, was injected into the whiskers or feet. Both pain stimuli led to EAE relapse. NeuN and cfos expression revealed that activated neurons expressed TRPV1 and/or Nav1.8 in trigeminal ganglions and blocking Nav1.8 or using TRPV1-deficient hosts suppressed the development of EAE relapse.[158] This led to the conclusion that sensory activation is involved in pain-induced EAE relapse. The mechanism by which pain induction led to relapse was then investigated. CD11b+ cells, specifically MHCII cells, were increased in the lumbar 5 (L5) spinal cord of EAE recovered mice following pain induction. Using parabiosis experiments, it was demonstrated that the MHCII cells originated from the peripheral monocytes in remittent hosts, whereas in relapse development, they were derived from the CNS. The MHCII cells led to activated helper T cells, which express cytokines, such as IL-6 and IL-17. Thus, these results indicate that expression of MHCII in L5 is important to the development of relapse through the accumulation of T cells.

Immune system components have also been confirmed in post-mortem brain tissue. One study, performed on tissue from patients with MS, examined the distribution of ectopic B-cell follicles via immunohistochemistry and morphometric analysis. B-cell follicles were detected in the meninges of patients and were found adjacent to lesions. This implies that B-cell follicles may have a pathogenic role in MS.[159]

Therefore, the chronic inflammation present in the CNS that characterizes MS is made clear by the data indicating the role of multiple immune cells including T cells, B cells, macrophages, and chemokines in the development and relapse of MS.

Diabetic neuropathy

Diabetic neuropathy (DN) is a common nerve disorder affecting approximately half of those with diabetes.[160] It is a result of high blood glucose, typically due to diet and the development of Type 2 Diabetes Mellitus. Symptoms vary depending on the neuropathy type and location but in general, numbness, tingling, and pain are often experienced first. Peripheral neuropathy is the most prevalent type and involves pain in the feet, legs, arms, and hands. Autonomic neuropathy affects the digestive system, bladder function, lungs, and eyes. It can also affect the heart and blood vessels, leading to changes in blood pressure. Proximal neuropathy affects the hips, buttocks, and legs. Finally, focal neuropathy can occur anywhere in the body and causes one or several nerves to become weak, leading to muscle pain.

Several studies have pointed towards inflammatory mediators in diabetes in the development of DN. Recently, using a rat model with nicotinamide-induced diabetes and streptozotocin (STZ)-induced neuropathy, one group measured gene expression of microRNA-146a (miR-146a). This was the first in vivo study investigating the role of miR-146a in DN. miR-146a is an innate immune system regulator[161] that is involved in multiple inflammatory diseases[162–165] and has been shown to be altered in diabetic patients.[166] Its two target adaptor proteins are IL-1 receptor associated kinase 1 (IRAK1) and TNF receptor-associated factor 6 (TRAF6). TRAF6 is important in NFκB activation, while both TRAF6 and IRAK1 are involved in TLR and IL-1 receptor signaling. These proteins as well as NFκB were measured in the sciatic nerve. Under normal conditions, a negative

feedback exists where TRAF6 and IRAK1 are decreased in response to an upregulation of miR-146a, which then ultimately leads to a decrease in NFκB activity. Expression of miR-146a and NFκB was significantly elevated, while TRAF6, a factor related to TNF receptor, was decreased. Further, NFκB activity and concentrations of TNFα, IL-6, and IL-1β were increased in the sciatic nerve of diabetic rats. Therefore, these findings in which there is an increase in NFκB activity instead of a decrease suggest that there is an error in the NFκB-miR-146a pathway, specifically the regulatory feedback, which may play a role in the development of DN.[167]

TNFα has specifically been examined using STZ-induced diabetic rats. Rats were inoculated with herpes simplex virus (HSV) vector expression p55 TNFα soluble receptor (vTNFsR). sTNF receptors act as TNF antagonists thus inhibiting pro-inflammatory effects of TNF. Diabetic animals with neuropathy who received the vector had decreased TNFα mRNA in the dorsal root ganglion (DRG). This illustrates that HSV-mediated expression of p55 TNF soluble receptor blocks the increase in TNFα mRNA expression seen in control animals. Along with this, immunohistochemistry demonstrated that vTNFsR treatment led to a decrease in TNFα protein levels in the diabetic rat DRG. Additionally, vTNFsR inoculated diabetic rats had less thermal and mechanical sensitivity compared to diabetic control rats.[168] Together, these data illustrate that TNFα is an important mediator of diabetic neuropathic pain.

It has been proposed that the inflammatory involvement seen in DN is due to damaged renal cells activating both the innate and adaptive immune system cells (see the study by Zheng and Zheng[169] for review). For example, the TNFα signaling induces macrophages take on the M1 type, T helper cells are upregulated and communicate with fibroblasts leading to fibrosis,[170] and tubular cells in the kidney can produce TGFβ to directly activate the immune system.[171]

These experiments highlight the role of inflammation in the pathophysiology of DN.

Conclusion

There is extensive pre-clinical and clinical research that exists examining the role of the immune system in chronic pain conditions. The existent data not only provide evidence of immune-related pathophysiology but also suggest promising avenues for treatment. However, detailed mechanisms or sufficient animal models are lacking in multiple cases. In order to fully treat patients suffering from diseases in which chronic pain is the primary symptom, novel animal models and in vitro approaches should be explored to provide sufficient pre-clinical data. Given that the available treatments involving the immune system and inflammation have proven successful, future studies should focus on immune signaling and the development of innovative treatments specifically targeting immune cells or receptors. Importantly, given the critical role that the immune system plays in various chronic pain conditions and the evident sex differences in the use of immune system cells,[71] it is critical that current and future work include both sexes to fully understand and treat these conditions.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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