Inflammation and its resolution and the musculoskeletal system

Jiri Gallo\textsuperscript{a,}*, Milan Raska\textsuperscript{b}, Eva Kriegova\textsuperscript{b}, and Stuart B. Goodman\textsuperscript{c}

\textsuperscript{a}Department of Orthopaedics, Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc, I.P. Pavlova 6, 779 00 Olomouc, Czech Republic

\textsuperscript{b}Department of Immunology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Hnevotinska 3, 775 15 Olomouc, Czech Republic

\textsuperscript{c}Department of Orthopaedic Surgery, Stanford University School of Medicine, 450 Broadway Street, Pavilion C, Redwood City, CA 94063-6342, USA

Summary

Inflammation, an essential tissue response to extrinsic/intrinsic damage, is a very dynamic process in terms of complexity and extension of cellular and metabolic involvement. The aim of the inflammatory response is to eliminate the pathogenic initiator with limited collateral damage of the inflamed tissue, followed by a complex tissue repair to the preinflammation phenotype. Persistent inflammation is a major contributor to the pathogenesis of many musculoskeletal diseases including ageing-related pathologies such as osteoporosis, osteoarthritis, and sarcopaenia. Understanding the mechanisms of inflammation and its resolution is therefore critical for the development of effective regenerative, and therapeutic strategies in orthopaedics.

Keywords

fibroblasts; inflammation; innate lymphoid cells; macrophages; neutrophils; proresolving mediators

Introduction

Inflammation contributes to all nontraumatic musculoskeletal diseases, causing pain and disability in millions of people worldwide \cite{1}. These orthopaedic and rheumatic diseases inflict an enormous burden for society and any health care system. At the same time, inflammatory processes play a critical role in tissue integrity and homoeostasis. In addition, the processes of healing and recovery after traumatic injuries, surgical procedures, infections and other adverse stimuli are driven by inflammatory pathways and mediators. Finally, inflammation is now considered one of the key pillars of ageing \cite{2} process involved in the pathogenesis of many ageing-associated musculoskeletal diseases. Together, these

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*Corresponding author. Department of Orthopaedics, Faculty of Medicine and Dentistry, Palacky University Olomouc and University Hospital Olomouc, I.P. Pavlova 6, 779 00 Olomouc, Czech Republic. jiri.gallo@volny.cz.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.
observations highlight the fact that inflammation plays a central position in orthopaedic diseases, their deterioration, and/or recovery.

Signs of inflammation are well known and have been used in clinical practice for more than 2000 years. These include *calor* (heat), *rubor* (redness), *dolor* (pain), and *tumour* (swelling). The fifth inflammatory sign, * functio laesa* (loss of function), was coined by Galen. From the pathologist’s and immunologist’s points of view, inflammation is the result of complex interactions between immune and inflammatory cells, their mediators, as well as regulators, and is a part of innate immune response. There are a number of differences between inflammation in health and in disease. One of the most important differences is that in a disease state, inflammatory cells and pathways continue to perpetuate inflammatory cycles regardless of the tissue structure; on the contrary, during inflammation in a state of health, the response is highly self-limited to a specific stimulus working with a “programme” to minimise tissue damage.

The aim of this review is to summarise current knowledge on inflammation with special attention to the control and resolution mechanisms of the inflammatory response in musculoskeletal tissues. A disturbance in these steps may result in excessive damage of the particular tissue as well as progression to persistent chronic inflammation. Examples of how inflammation affects the musculoskeletal system are presented. Moreover, the importance of resolution inflammation in orthopaedic disorders is underlined, highlighting opportunities for therapeutic intervention in this field.

**Current concept of inflammatory mechanisms**

Inflammation is a protective response driven in a tissue compartment by a specific set of immune and inflammatory cells with the aim of restoring its structural and functional integrity after exposure to an adverse stimulus. Detrimental variables can be of both intrinsic and extrinsic origins [3]. The first are variables deviated from particular tissue homoeostatic values (biological, biochemical, biomechanical), whereas the second are extrinsic (pathogens, toxins, allergens) in relation to a particular inflamed tissue compartment [4].

In order to detect microbial and viral invasions, cells are equipped with receptors called pattern recognition receptors (PRRs). Among the PRRs, the Toll-like receptors (TLRs) have been studied most extensively. They recognise conserved structures on pathogens, termed pathogen-associated molecular patterns (PAMPs). Upon PAMPs engagement, PRRs trigger intracellular signalling cascades leading to activation of complex host inflammatory response.

Also, host biomolecules produced by damaged or stressed cells can initiate and perpetuate a noninfectious inflammatory response. After a signal of danger and/or damage [danger (danger)-associated molecular patterns (DAMPs)] is recognised by particular sensor-bearing cells, the inflammatory response programme is activated, leading to local increased production of proinflammatory cytokines/chemokines by innate immune cells (Figure 1). Although a degree routine surveillance and “inflammatory readiness” occurs in all tissues in order to maintain an appropriate functional status [3], the immediate and heightened
response activates innate immune cells as well as the inflammasome and other mechanisms to preserve homeostasis [5]. The inflammasome is a protein complex that archives premature forms of the proinflammatory cytokines [interleukin (IL)-1β and IL-18] that are released after stimulation by caspase-mediated cleavage.

The onset of inflammation is associated with a specific reaction of the microcirculation enabling the efflux of plasma proteins including antibodies, complement, and other mediators. Moreover, chemokines attract circulating neutrophils, monocytes and other immune/regulatory cells to the site of injury to counteract the causative agent in the affected tissue. The migratory cells together with tissue-resident and accessory cells direct the inflammatory processes towards resolution after their task is successfully accomplished. In pathologic chronic inflammation, the inflammatory processes are not resolved, and active inflammation continues in a dysregulated fashion.

Tissues developing chronic inflammation exhibit pain and swelling, and histologically develop a typical cellular infiltrate. Distinct subsets of leucocytes are distributed across the inflamed tissue characterising the type of chronic inflammation more precisely. They may manifest organised into well-defined lymphoid-like structures as in rheumatoid diseases, for instance [6]. Increased secretion of synovial (pseudosynovial) fluid accompanies chronic inflammation at the site of a joint replacement (an artificial joint); dry inflammation can also occur. Similarly, but to much less extent, synovial fluid can accompany the inflammation of synovial and fibrous tendon sheaths. In this line, it is therefore crucial to know what underlies the failure to resolve acute inflammation, leading to chronic inflammation.

Moreover, some studies associate the chronic systemic inflammatory state also with ageing (“inflammageing”). It is understood as low-grade inflammation without concomitant infection and/or systemic inflammatory disease [7]. The mechanisms behind inflammageing have not been fully elucidated yet. Despite this, we believe that the mechanisms are complex and closely associated with genetic and epigenetic factors [8], mitochondrial dysfunction [9,10], and small metabolic and homeostatic dysregulations leading to a maladaptation to normal stresses, thus resulting in macromolecular/cell/tissue damage. In this way, chronic low-grade inflammation could contribute to many ageing-associated orthopaedic and rheumatological pathologies such as osteoporosis [11], osteoarthritis [12], and sarcopaenia [13].

Cells contributing to inflammation

There are a number of cells fuelling an inflammatory response (Table 1). Some of these exhibit key immunologic functions; others are also essential but serve as accessory ones. Here, we briefly mention the main populations as neutrophils, macrophages, and lymphocytes as well as mast cells and their involvement in orthopaedic disorders.

Neutrophils—Neutrophils are powerful defences driving the antibacterial inflammatory response. Besides their key role in infection, they also participate in a wide range of nonbacterial inflammations.
Neutrophils are continuously formed within the bone marrow during haematopoiesis under the influence of a spectrum of cytokines and chemokines and the specific environment. Their amount and turnover in the bloodstream as well as in the target tissues are tightly balanced via feedback loops regulating the production and survival of neutrophils. Circulating neutrophils are quiescent; they are fully primed only after entering the infected or inflamed tissue site. Local activation of primed neutrophils occurs either by phagocytosis of opsonised bacteria or by frustrated phagocytosis [14]. The signs of neutrophil activation include a release effector proteins (cytokines, chemokines, cytotoxic substances, including antimicrobial proteins, etc.), exhibiting phagocytic capabilities; generation of reactive oxygen species; or production of neutrophil extracellular traps (NETs) [15]. In the case of rheumatoid arthritis, neutrophils migrate into joints in response to CXCL8 signalling, which promotes neutrophils to release NETs [16]. Importantly, neutrophils are important effector cells that can also induce “bystander” tissue damage besides their positive mission, i.e., elimination of pathogens. Therefore, neutrophils have to be tightly regulated in order to avoid collateral tissue damage. There are numerous interactions between neutrophils and tissue resident (parenchymal cells, fibrocytes, myocytes, osteoclasts, osteoblasts, etc.) and accessory cells (resident macrophages, fibroblasts, other stromal cells, bone marrow cells, etc.). A failure to regulate the number, survivorship, and/or functions of neutrophils can contribute to chronic inflammatory diseases. Details are described in a recent review [15].

**Macrophages**—Macrophages are now understood as diverse, polyfunctional, and plastic cells providing vital immunologic roles in almost all tissues and organs. In the orthopaedic context, the crucial role of macrophages in the development of numerous inflammatory and noninflammatory diseases is emphasised.

There are at least two distinct populations of macrophages, distinguished by conditions of their development, and maintenance [17]. The first group consists of tissue-resident macrophages originating from prenatal precursors and further maintaining their numbers by self-renewal. They are integral components of the host tissue, exhibiting a wide range of local adaptations (tissue- and organ-specific) and providing a number of essential services. The tissue-resident macrophages are tightly regulated with tissue-selective transcriptional control, and, importantly, they possess some stem cell-like properties [18]. The second group of macrophages originates from haematopoietic stem cells in the bone marrow belonging to monocyte-derived macrophages [19]. Monocyte precursors circulate permanently in the bloodstream, always ready for rapid “on demand” recruitment based on particular tissue requirements. There can be a difference between tissues in terms of “embryonal” and “haematogenous” macrophages. In relation to inflammation, macrophages can exhibit proinflammatory (often termed M1 macrophages having IL-12 high, IL-23 high, IL-10 low) and anti-inflammatory, proresolving, or reparative characteristics (M2 macrophages, having reverse characteristics compared to M1: IL-12 low, IL-23 low, IL-10 high, CD206 high) [20]. M1 and M2 macrophages represent the two extremes of a spectrum; many macrophages have mixed characteristics (Figure 2).

The “haematogenous” macrophages communicate with resident-tissue macrophages via a set of regulatory molecules. Both groups interplay with other immune and inflammatory cells,
especially regulatory T (T\textsubscript{reg}) cells that are dominant producers of IL-10. Several recent papers describe the roles of macrophages in health and disease in detail [3,19,21–24].

**Mast cells**—Recently, a pathogenic contribution of mast cells to a number of diseases including rheumatoid arthritis and even osteoarthritis was demonstrated [25,26]. Mast cells have been also identified in periprosthetic synovial-like membranes associated with aseptic loosening and septic failure even though their number was low [27,28].

Mast cells are effector immune cells contributing importantly to the first line of defence against invading pathogens. In addition, these cells play an important role in autoimmune diseases and allergic response. These cells have a set of specific surface and intracellular markers [29]. Cell—cell interactions, growth factors (e.g., stem cell factor, transforming growth factor \(\beta\)), and specific cytokines (IL-3, IL-4) create a vital microenvironment in synovium, tendon sheaths, and synovium-like tissues critical for mast cell activity and survival.

Mast cells can be activated by PAMPs, DAMPs, and specific cytokines, and also by cell–cell interactions because they firmly reside in their local tissues [30]. In response to activation, mast cell plasticity confirms their ability to alter their phenotype and function depending on the type of danger, tissue damage signals, and resulting transcription programme. At least two types of mast cells are ascribed, depending on the expression of specific proteases: (1) tryptase-only positive cells, MC\textsubscript{T}; (2) tryptase–chymase double-positive cells, MC\textsubscript{TC} [29,31]. Well known is their prompt release of strong mediators of inflammation (histamine, proteases, heparin, etc.) via a mechanism of degranulation. However, mast cells can synthesise a wide range of cytokines and growth factors de novo within several hours. Thus, mast cells regulate the functions of many cell types, such as dendritic cells, macrophages, T cells, B cells, fibroblasts, eosinophils, endothelial cells, and epithelial cells. Their role is still underscored in musculo-skeletal diseases.

**Lymphocytes**—Lymphocytes are a heterogeneous group of cells that are traditionally subdivided into T cells, and B cells and natural killer (NK) cells. All of these subgroups are responsible for a specific array of immune responses, and thus also heavily contribute to local tissue inflammation and its resolution. Here, we review lymphocytes and innate lymphoid cells (ILCs), briefly and with a great simplification.

T cells can be divided into cytotoxic, regulatory, helper, and memory subsets based on their distinct functions as well as their subtle phenotypic characterisations. In relation to local tissue homeostasis, tissue-resident memory T cells initiate a rapid and highly effective immune response against previously contacted pathogens. Details on the formation and regulation of this important group of T lymphocytes have been published elsewhere [32]. As is typical of many resident cells, the response differs according to the host inflamed tissue (i.e., synovial tissue, lung, skin, gout). Pathologically activated or dysregulated tissue-resident T cells contribute to tissue-specific inflammatory diseases.

T cell receptor signalling also plays an essential role in differentiation and function of T\textsubscript{reg} cells. T\textsubscript{reg} cells substantially contribute to the resolution of inflammation. They are pivotal to
controlling effector T cell proliferation and activation. They control self-reactive T cells, maintain immunological self-tolerance and homoeostasis, and govern the interplay between innate and adaptive immunity. A decrease in the number of T<sub>reg</sub> cells or a loss of their function leads to chronic autoimmune diseases. Recently, important plasticity of the T cell group—rather context-dependent—has been reported, diminishing a clear borderline between distinct T cell subsets, somewhat confusing our knowledge of the role of particular T subsets in the processes of inflammation and tissue homoeostasis. For instance, some T<sub>reg</sub> cells are producers of interferon (IFN) gamma, which means they could act, for instance, as effector T cells during nonresolving inflammation [33].

Recently, it has been shown that ILCs play a central role in the innate immune response and tissue remodelling [34]. These cells react promptly to signals from damaged tissues organising the tissue response to the original insult [35]. Several distinct ILC populations have been described depending on their origin, development, and postdevelopment signalling through a set of receptors/cytokines. One group, natural killer (NK cells, are defined by a strong production of IFN-γ [36]. IFN-γ initiates a very rapid and influential inflammatory pathway and orchestrates the response to a wide range of stimuli. Additionally, NK cells exhibit direct cytotoxic activity contributing in this way to the elimination of bacteria- or virus-infected cells and signalling between innate and adoptive immunity.

B cells participate also in the complex mechanism of inflammation despite that their primary role of producing antibodies against invading pathogens. B cells also interact with and activate other cells such as T cells, NK cells, and fibroblasts [37]. B regulatory cells are also important for achieving and maintaining immune tolerance in chronic inflammation [38].

**Sensors detecting danger/damage stimuli**

Specialised sensors (receptors) continuously search for signs of tissue damage and abnormal signals (Table 2). Those devoted to identification of microbial and injury danger are well understood, whereas most of those dedicated to sensing an abnormality in regulated tissue variables remain unknown. Detection of microorganisms is realised mainly through germline-encoded PRRs that control the extracellular environment, whereas the intracellular ones search for intracellular signals. Most PRRs belong to one of five families based on protein domain homology [39]. These are TLRs, C-type lectin receptors, NOD-like receptors, RIG-I-like receptors, and AIM2-like receptors (ALRs). Membrane-bound receptors are some TLRs and C-type lectin receptors; intracellular cytoplasmic receptors are NOD-like receptors, RIG-I-like receptors, and AIM2-like receptors and some TLRs.

PRRs do not usually function as responding molecules alone; they require adaptor molecules to transmit further signals. In addition, ligand-dependent receptors have to perform movement to activate an adaptor molecule to initiate signal transduction. These details are characterised elsewhere [39].

**Molecules mediating inflammatory communication**

The inflammatory response is orchestrated by numerous proinflammatory cytokines and chemokines. These molecules regulate a wide range of events, for example, the migration,
proliferation, and function of inflammatory and immune cells (phagocytic, secretory, etc.),
the switch of resident-tissue macrophages between senescence to inflammatory status and
also relevant changes in the micro-circulation [3]. Chemokines influence the recruitment of
circulatory monocytes and neutrophils and other cells to inflamed tissues. These cells
govern, among others, the processes in which the initiator of inflammation can be eradicated
with or without the help of the adaptive immune system. There is a growing body of
evidence that chemokines also contribute to tissue regeneration and repair. Importantly, the
inflammatory mediators are both antagonistic and dominant over homoeostatic molecules
mainly because of the conflict with the goals of homoeostatic and inflammatory processes
[40].

Many chronic diseases in various organs are linked to the persistence of chronic
inflammation. Regardless of the initial trigger stimulus, the timely termination of the
inflammation process is critical to prevent unwanted tissue damage.

Resolution of inflammation

Inflammation is an unstable state that either resolves or persists [41]. Therefore, the
resolution of inflammation is an essential part of the host response to a danger/damage
stimulus after it has been eliminated, or after the tissues have adapted to the stimulus (Figure
3). The reasons for the resolution could be as follows: avoiding unnecessary tissue damage,
reduction of energy, cellular and homoeostatic costs associated with inflammation and tissue
damage, pain relief, remodelling, regeneration, and restoration of function.

Ilya Metchnikoff was among the first to observe macrophages phagocytosing and digesting
neutrophils, and conjoined these processes with the resolution of inflammation [42].
Currently, our understanding of the resolution is much more complex. Above all, it is an
active process encountering specific cells, mediators, hormones, and also neural signals
[43,44]. Together, these agents achieve an active clearance of inflammatory cell infiltrates,
ideally with a minimum of collateral tissue damage (in cases directing towards resolution).
However, it is still not known why some danger/damage stimuli in some tissues/organs
develop chronic (i.e., persistent) inflammation whereas others can manage resolution
without excessive and long-term tissue damage. The examples of the former group are
chronic joint inflammatory diseases regardless of their aetiology.

Whether the mechanisms underlying the resolution of inflammation are general (“one size
fits all”) or include tissue specific programmes is still unknown [42]. It seems more plausible
that the resolution phase of inflammation is directed by means located in the inflamed
tissues rather than by a general anti-inflammatory programme activated equivocally for all
types of inflammation and regardless of their cause, type of the immune response,
anatomical site, etc.

Key players: cells contributing to resolution of inflammation

The resolution of inflammation is a multifaceted process orchestrated by several groups of
cells in concert with humoural and neural regulatory facilities. The tasks seem to be at least
two: (1) to stop inflammatory processes (i.e., removal of cells and downregulation of
inflammatory signalling) and (2) to renew the original structure of the inflamed tissue. In order to accomplish this, immune and nonimmune cells responsible for resolution mechanisms of the inflammatory response express a specific set of genes coding for substances with proresolving and recovering effects. There is also accumulating evidence emphasising the role of multiple cell–cell communications occurring in inflamed tissues. Neutrophils, lymphocytes, eosinophils, and specific resident-tissue macrophages are among the most important immune cells, whereas fibroblasts, stromal cells, and endothelial cells are examples of nonimmune cells participating in the resolution and renewal of the tissue damaged by an inflammation [42,45].

There are a number of proresolving mechanisms. For instance, the chemokine gradient required for attracting cells from the intravascular compartment to the site of inflammation is gradually diminished via either a cell density-dependent sensing mechanism or accumulation of neutrophil-related products (such as aggregates of NETs and proteases) leading to deactivation of inflammatory chemokines/cytokines. The crucial influx of neutrophils is reduced by annexin A1, once sufficient numbers of neutrophils are achieved, acting in an autocrine and/or paracrine fashion. Annexin A1, a protein with strong proresolving efficacy, is produced by neutrophils and other cells and induces neutrophil apoptosis and macrophage reprogramming towards a resolving phenotype, stimulates macrophage efferocytosis, and reduces neutrophil–endothelial interactions [46]. Details on the known mechanisms of resolution are described elsewhere [43].

**Neutrophils**—Besides involvement in infections and inflammation, these cells are highly involved also in the resolution and healing processes. Patients with a depletion of neutrophils exhibit wound healing disturbances; this finding, together with experimental studies, consistently supports the concept of “resolution neutrophils.” A number of studies examined the mechanism by which neutrophils exhibit their “anti-inflammatory and homoeostatic” effects. Neutrophils are able to downregulate inflammatory pathways by releasing proteases that affect chemokine and cytokine gradients or by the release of anti-inflammatory proteins. Details on anti-inflammatory and homoeostatic functioning of neutrophils are described in recent studies [15,45].

**Resident tissue macrophages**—Tissue macrophages also play a key role in the resolution stage of inflammation [47]. Resolution of inflammation is associated with an active switch of macrophages from a proinflammatory to anti-inflammatory phenotype. These cells remove the apoptotic cells and express a wide range of anti-inflammatory proteins and proresolving mediators, and interact directly with other cells (cell–cell communication) thus contributing to resolution of inflammation.

**Lymphocytes**—Many processes associated with the resolution of inflammation are governed by T regulatory cells and other T cell subpopulations. Their effect is mediated by cell–cell interactions, synthesis of anti-inflammatory cytokines, and proresolving mediators. Details on their role in the mechanisms of resolution and return to homoeostasis are deeply described elsewhere [48].
**Fibroblasts and other stromal cells**—Stromal cells encompass numerous cell types occurring inside a tissue/organ such as fibroblasts, endothelial cells, pericytes, epithelial cells, and anatomically specialised cells such as astrocytes. These cells play many roles including architectural, regulatory, and supporting ones [41]. Most importantly, these cells reconstruct the shape and function of the normal host tissue at the stage of resolution of inflammation.

Fibroblasts are ubiquitous cells identified by their morphology, ability to adhere to a plastic substrate, and lack of other cell lineage markers [41]. They undergo a similar life cycle as do macrophages, beginning as circulating fibrocytes, colonising an appropriate tissue, and transforming into tissue-resident cell type fibroblasts. They are primarily responsible for synthesis and remodelling of extracellular components of the distinct tissue. Therefore, they differ depending on their anatomical site (synovium, bone marrow, liver, lung, etc.). Synovial fibroblasts express a number of molecules under normal and inflammatory conditions such as adhesins, cytokines, chemokines, as well as matrix metalloproteinases and cathepsins [41].

In relation to the resolution of inflammation, fibroblasts (as well as other stromal cells) exhibit strong anti-inflammatory and proresolving capacities and are considered to be crucial to tissue regeneration and achievement of original tissue appearance, structure, and function (Figure 4). They appear to remember a “tissue map” in terms of cell differentiation, positional, and survival information of a particular tissue site where the inflammation is ongoing. Alternatively, dysfunction of fibroblasts/fibrocytes can result in pathologic scar formation, excessive tissue/organ fibrosis, and chronic inflammation associated with loss of function of the damaged tissue [6]. A specific role has been attributed to fibrocytes that contribute to chronic inflammation in systemic inflammatory diseases [49].

**Eosinophils**—Eosinophils are innate host defence/homoeostatic cells with a wide range of immune and regulatory functions. They have the ability to synthesise numerous cytokines and chemokines including those stored for subsequent release. Traditionally, they have been associated with the allergic hyperactive response. However, recently these cells have been positioned among those of local immune and remodelling/repair activities (local immune and/or remodelling/repair concept). Therefore, an accumulation of eosinophils and their function are vital components of the resolution stage of inflammation, rebuilding the damaged tissue, as well as achievement/maintenance of local tissue homoeostasis and health [50].

**Mediators governing the resolution phase of inflammation**

In addition to various cells, numerous humoural mediators also contribute to the resolution of inflammation (Table 3). The resolution mediators are produced both locally and systemically. The former group consists of anti-inflammatory cytokines and chemokines, lipid mediators of resolution, adenosine, micro-RNAs, nitric oxide, and inhibitors of PRRs and DAMPs, whereas the latter group of regulators consists mainly of steroids. For instance, activated inflammasomes release among others a danger signal, high mobility group box 1 protein (HMGB1), which is a ligand for the receptor for advanced glycation end products
(RAGE). Thus, neutralisation of HMGB1 and/or RAGE receptors should lead to reduced inflammation [51].

Importantly, neuroregulatory circuits contribute to the resolution of inflammation and tissue renewal [44]. Conceptually, those substances should exhibit not only a wide array of activities targeting key inflammatory agents but also, they should return a tissue to a state of homoeostasis without fibrosis, excessive scar formation, or other tissue maladaptation. Their anti-inflammatory and homoeostatic contributions are evident from early onset of an inflammation.

Proresolving mediators are the critical components of the resolution phase. They are lipid mediators (lipoxins, resolvins, protectins, and maresins), proteins (annexin A1), peptides, gaseous mediators (e.g., hydrogen sulphide, carbon monoxide), adenosine, neuromodulators (neuro-transmitters, neuropeptides), etc. [43]. The majority of these molecules exert their effects via specific G-protein triggering proresolution pathways rather than serving as blockers of the proinflammatory signals [43].

The proresolving mediators differ from the anti-inflammatory triggers in the following abilities [42]: (1) they neutralise or counter regulate molecules attracting leucocytes and other immune cells to the site of inflammation; (2) they stimulate the recruitment of anti-inflammatory monocytes to the site of inflammation; (3) they activate macrophages to the clearance of the tissue affected by inflammation (cells, tissue debris, etc.); (4) they shift the specialised signalling inflammatory pathways towards anti-inflammatory ones; (5) they affect adenosine triphosphate metabolism; and (6) they dampen the clinical signs of inflammation, especially pain and swelling.

Recently, it has been demonstrated that classical resolution may not be the end of the local immune response; rather, there is a third “postresolution” phase [52]. In the postresolution phase, the key processes include the clearance and apoptosis of infiltrating inflammatory cells, alterations in macrophage phenotype into prohealing phenotype, and orchestrating other fundamental processes leading to full tissue regeneration [52,53].

**Persistence of inflammation: why is every inflammatory event not resolved?**

Persistence of inflammation concerns the lack of balance between the recruitment and removal of inflammatory cells, regulation of inflammatory/anti-inflammatory pathways, and less efficient homoeostatic mechanisms.

It is unlikely that a single molecule/agent is responsible for the persistence of inflammation in each tissue/organ/clinical situation. Rather, distinct mechanisms related to the particular tissue/organ/disease could play a role in persistence of inflammation and the development of a disease. Defects can be located at the level of insufficient switch-off of proinflammatory chemokine/cytokine signalling. Nonresolution could be associated with an inability to effectively inhibit signalling from PRRs and/or DAMPs. In relation to the persistence of inflammatory cellular infiltrate, an inappropriate triggering of apoptosis may play a role.
Some epigenetic factors might also contribute to longer survival as well as inappropriate production of survival factors such as type I IFN [6].

Knowledge of the mechanisms of resolution of inflammation is critical in clinical practice. For instance, chronic synovitis plays an important role in many patients suffering from osteoarthritis [54]; however, we know very little about the mechanisms of resolution of inflammation and synovial restoration in this particular disease [12]. Thus, nonsteroidal anti-inflammatory drugs are routinely prescribed regardless of the phase of inflammation and individual disease phenotype. However, their usage (especially the so-called cyclooxygenase-2 inhibitors) could interfere with a late-phase synthesis of proresolving mediators, whereas in the early phase, they may have a beneficial effect owing to elimination of inflammatory agents [45].

**Future developments**

Currently, we are realising a more profound and complete understanding of pathogen-mediated inflammatory responses as well as a number of sterile orthopaedic/rheumatic diseases. In addition, new molecules that function in the resolution of inflammation are being discovered and even synthesised with the aim of controlling excessive inflammation and preventing the development of chronic inflammation. Synthetic biologists have started the development of engineered cells finely tuned for therapy of chronic inflammatory diseases [55]. This “device” should combine a receptor (sensory) module with an effector one using chemical “wires” [56]. However, a number of challenges are associated with this approach, and many gaps remain in our knowledge of distinct tissue/organ-specific inflammation and its resolution. That is especially true for orthopaedic inflammatory diseases. As a result, further studies should address this lack of information on distinct articular and extra-articular orthopaedic diseases with inflammatory contribution. These models could be appropriate to persistent synovitis [57], tenosynovitis, tendonitis, myositis, sarcopaenia [58], osteoarthritis [59], osteomyelitis [60], osteoporosis [61], and inflammatory implant-related pathology [62].

In relation to the cells contributing to inflammation and resolution, research in circulating fibrocytes as well as ICLs and modulation of macrophage properties might open new avenues to further progress in the therapy. For instance, tenocytes and ligament fibrocytes could be utilised in tissue engineering related to meniscus, artificial ligaments, and fibrocartilage [63].

Inflammation and its resolution depend in part on the location and properties of the specific tissue involved. Thus, it seems logical to expect that some critical processes such as removal of inflammatory neutrophils or exhausted macrophages are tissue-dependent. Therefore, critical checkpoints should be analysed in relation to major orthopaedic tissues. Different strategies will probably be effective in the bone compartment during specific anti-osteoporotic interventions, whereas others will be efficient for skeletal muscle weakness associated with sarcopaenia. In the former, proresolving macrophages could deliver the anabolic factors as well as interfere with inflammation-induced bone loss and restore tissue microarchitecture [64]. Inflammation-induced bone loss could be also affected by targeting...
nuclear factor kappa B transcription factor [65]. In the latter, an interfering with muscle stem cell–niche interactions could retard the architectural and functional changes in the skeletal muscles [58].

Currently, nonsteroidal anti-inflammatory drugs and locally delivered corticosteroids constitute the only anti-inflammatory weapons in an orthopaedic surgeon’s hands. However, an exciting new class of proresolving molecules have been tested in the lung, skin, or gouty inflammations. Another very promising factor is the therapeutic targeting of pathways that initiate proresolution processes (removal of the stimuli, dampening of proinflammatory signalling, and enhanced mediator catabolism), which may improve the outcome of inflammatory musculoskeletal disorders, as shown already in animal models. However, the heterogeneity of inflammation, disease-specific factors, and tissue-site differences in resolution pathways should be taken into account when designing the suitable “pro-resolution approach.” Therefore, translational research that would test the efficacy and timing of these molecules and targeting the proresolution pathways in the most frequent/debilitating orthopaedic diseases is highly expected.

Conclusion

Dysregulated or persistent inflammation is a major contributor to the pathogenesis of many orthopaedic diseases. By contrast, the inflammatory reaction is an essential tissue response to extrinsic and intrinsic damage. Inflammation is a very dynamic and expensive process, especially in terms of complexity and extension of cellular and metabolic involvements. The main aim of the inflammatory response is to eliminate the pathogenic initiator with a limited collateral damage of the inflamed tissue followed by a complex tissue repair to the preinflammation phenotype. Therefore, the resolution of an inflammation is a vital process. It includes the inhibition of PRRs, DAMP stimulation, neutralisation of inflammatory cytokines/chemokines activity, inhibition of neutrophil tissue infiltration, as well as removal of apoptotic neutrophils together with activity of proresolving and resolving mediators. Simultaneously, tissue damage caused by the invasive agent and/or the inflammatory response should be repaired to the preinflammatory phenotype.

Understanding the mechanisms of resolution is critical for development of effective anti-inflammatory strategies that could be translated in the clinical practice in orthopaedics. In addition, the mechanisms underlying the restoration of tissue homeostasis could be used in tissue engineering and other parts of regenerative medicine in orthopaedics and traumatology. Taken together, this knowledge could open new avenues to treatment of musculoskeletal diseases.

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Mechanisms of macrophage sensing of apoptotic bodies and various external and internal danger signals and intra-cellular pathways involved in macrophage response. Macrophage sense “find me,” DAMP, and “eat me” stimulators by several classes of receptors such as GPCR for “find me signals”; TLR, RAGE, NLR, CLR, TNF-R, RLR, and HMGB for DAMP signals; and several various receptors allowing phagocytosis of microbial pathogens or “eat me” signals for apoptotic cells. Receptors recognise various ligands as exemplified in Table 2. Binding of “find me” signals to GPCRs (7-transmembrane domain-containing protein) such as P2Y2, G2A, S1P-R, and CX3CR induces conformational changes allowing the coupling of the receptor to the three subunits of G protein and promotes the exchange of GDP for GTP on the α subunit. As a consequence, chemotaxis and other biological responses are induced, including cell activation, survival, cell–cell interaction, and adhesion.

Extracellular, endosomal, and intracellular PRR sense various microbial and endogenous stimuli specified in Table 2, which could trigger the production of inflammatory signals represented mostly by cytokines. Extracellular PRR receptors such as TLR-1, -2, -6, -5, -11 stimulated by bacterial lipoprotein, flagellin, etc., trigger the activation and translocation of transcription factors NF-κB, AP-1, and CREB to the nucleus. Described pathways involved in the activation of NF-κB, AP-1, and CREB are stimulated also by LPS, calcium- and zinc-binding proteins S100 [predominantly found as calprotectin (S100A8/A9)], and HMGB1, all sensed by RAGE, and upon ligation of TNF-α to its receptor (TNF-R). LPS also activates its primary extracellular receptor, TLR-4, which could contribute to the activation of IRF3, a transcription factor involved in the regulation of expression of several proinflammatory cytokines. After endocytosis of microbial pathogens or apoptotic cells, DAMPs stimulate membrane-anchored TLR-3, -7, -8, -9, but some DAMPs could be released into cytosol, for example after endosomal rupture, and could interact with NOD receptors or with inflammasomes. Inflammasomes represent a heterogeneous group of protein complexes forming either NLRP, NLRC, or AIM2. After interaction with the LRR domain, selected
DAMPs induce conformational changes leading to NLRP, NLRC caspase-1 activation allowing cleavage of cytokine precursors pro-IL-1β and pro-IL-18 to active IL-1β and IL-18, which are subsequently released from the cell. NODs are activated by bacterial flagellin, RNA, muramyl dipeptide (MDP). NLRP and NLRC are activated in a biphasic manner consisting of (1) their transcription initiated with contribution of transcription factors NF-κB and probably AP-1 and IRF3, and (2) their subsequent activation through various DAMPS. Bacterial and viral nucleic acids could stimulate inflammation through TLR-3, -7, -8, -9, which signal through IRAK4 and IRAK1 to activate TRAF 6 but also TRAF 3 and subsequently IRF7, transcription factor involved in the regulation of transcription of several members of the IFN-α family, and other transcription factors mentioned above. Nucleic acids could also stimulate inflammation through cytosolic helicases: RIG-I and MDA5 signalling through mitochondrial membrane-associated adaptor IPS-1 involved in activation of TBK1/IKKe, NF-κB, and MAPK-depended AP-1. Double-stranded cytosol-localised DNA could also be sensed by DNA-binding protein DAI signalling through endoplasmic reticulum-associated stimulator of interferon genes (STING), which interacts with TBK1/ IKKe. Furthermore, HMGB1–DNA complexes released from damaged cells could be captured by surface-exposed RAGE; endocytosed and DNA within endosome is recognised by TLR7 or TLR9. Similarly, autoantibodies recognising self-DNA or -RNA could facilitate endocytosis of antibody–DNA complexes for endosomal TLR7 and TLR9 recognition. All the above pathways merge on a few transcription factors CREB, NF-κB, AP-1, and IRF3 involved in transcription of proinflammatory cytokines IFN-β1, IL-6, IL-12, TNF-α, GM-CSF, IL-8, IL-10, IL-33, as well as IL-1β and IL-18. The fifth depicted transcription factor IRF7 is responsible for transcription of several members of the IFN-α family. Finally, phagocytes engulf dead cells or apoptotic bodies through their recognition by virtue of a characteristic “eat me” signal exposed on their surface. A typical “eat me” signal is phosphatidylserine, a cell plasma membrane component that is kept in healthy cells exclusively on the inner leaflet of the lipid bilayer, whereas in apoptotic cells it is exposed on the cell surface. Secreted proteins MFG-E8 and Gas6 bind phosphatidylserine and serve as bridging molecules between apoptotic cells and macrophages surface-exposed receptors Tim-4, BA11, and Stabilin-2. These receptors activate Rac1 through (CrkII, Dock180, Elmo, and GULP) finally activating the engulfment of apoptotic cells. AIM2 = absent in melanoma 2; AP-1 = activator protein-1 (complex of transcription factors cJun, cFos); BA11 = brain-specific angiogenesis inhibitor 1; CLR = C-type lectin receptor; CREB = cAMP responsive element binding protein 1; CX3CR = CX3C chemokine receptor, FrC = fractalkine receptor; DAI or ZbpI = DNA-dependent activator of IRF; DAMP = danger-associated molecular patterns; Gas6 = growth arrest-specific 6; GM-CSF = granulocyte macrophage colony-stimulating factor; GPCR = G protein coupled receptor; HMGB1 = high mobility group box 1; IL = interleukin; IPS-1 = mitochondrial antiviral-signalling protein; IRAK = interleukin-1 receptor associated kinases; IRF = interferon regulatory factor; LPS = lipopolysaccharide; MAPK = mitogen-activated protein kinase; MDA5 = melanoma differentiation-associated protein 5; MDP = muramyl dipeptide; MFG-E8 = milk fat globule EGF factor 8; NF-κB = nuclear factor kappa B; NLR = NOD-like receptor; NLRC = NACHT-, LRR- and Caspase-recruitment domain (CARD)-containing protein; NLRP3 = NAIP, CIITA, HET-E and TP1 (NACHT), Leucine-rich repeat (LRR)- and Pyrin domains (PYD)-containing protein; P2Y2 = purinergic receptor; RAGE = receptor of advanced glycation end products; RIG-I =
retinoic acid-inducible gene I; RLR = RIG-I-like receptor; S1P-R = sphingosine-1-phosphate receptor; Tim-4 = T cell immunoglobulin and mucin-domain-containing molecule 4; TLR = Toll-like receptor; TNF-R = tumour necrosis factor receptor; TRAF = tumour necrosis factor receptor associated factor; TRIF = TIR-domain-containing adaptor inducing interferon-β.
Figure 2.
Current view of macrophage phenotypical versus functional differentiation and involvement in inflammation and resolution. Resident tissue macrophages ensure various tissue-specific accessory functions (osteoclasts perform bone resorption and remodelling, microglia ensure synaptic pruning) as well as tissue repair and host defence functions. Thus, in addition to homoeostatic variables sensed and modified by tissue resident macrophages, as a part of their unique tissue-specific accessory functions, tissue resident macrophages sense bacterial infection, dead cells, extracellular matrix, tissue microenvironment, etc., through several receptors, as specified in Table 2. The tissue repair and host defence functions are performed only on demand when the challenge is sufficiently small. When the challenge (infection or injury) exceeds the resident tissue macrophages capacity, they recruit specialised accessory cells including inflammatory monocytes and neutrophils by production of appropriate chemokines, and they could probably increase the number of acting macrophages by locally stimulated proliferation, too. Thus, local inflammation is initiated as a predominant first step response to infection or injury. The inflammation may be later resolved with contribution of opposite-polarised macrophages. Between the above two extremes, there is a continuum of functional intermediates contributing to wound healing, neovascularisation, tissue remodelling and repair, etc. Macrophage activation and polarisation to proinflammatory M1 (classic) or anti-inflammatory M2 (alternate) phenotypes or several functional intermediates could be achieved in vitro by IFN-$\gamma$, LPS, glucocorticoids, TGF-$\beta$, IL-10, IC, and IL-4.

Furthermore, several other factors contribute to macrophage activation and polarisation in vitro...
**vivo** including efferocytosis of apoptotic cells formed at the inflammation focuses, cytokines, lipid mediators derived from ω-3 eicosapentaenoic acid (ω-3 EPA) and ω-3 docosahexaenoic acid (ω-3 DHA) as well as the miRNAs and long noncoding RNAs (lncRNAs). DAMP = danger-associated molecular pattern; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; GM-CSF = granulocyte macrophage colony-stimulating factor; IC = immune complex; IFN = interferon; IL = interleukin; LPS = lipopolysaccharide; M-CSF (CSF-1) = macrophage colony-stimulating factor; miRNA = short noncoding RNAs of approximately 21–23 nucleotides in length; PDGF = platelet-derived growth factor; TGF = transforming growth factor; TNF = tumour necrosis factor; VEGF = vascular endothelial growth factor; Ym-1 = chitinase 3-like 3.
Figure 3.
Mechanisms involved in initiation, perpetuation, and resolution of inflammatory responses. The immune response is initially induced by triggers (inducers) DAMPs and PAMPs sensed by tissue resident macrophages and other cells such as mast cells and NK cells (Ia) to resolve the trigger locally. If the injury or infection is not resolved locally by resident cells continuous increase in production of proinflammatory cytokines, chemokines, vasoactive amines, and proinflammatory lipid mediators contribute to attraction of inflammatory monocytes and neutrophils from peripheral circulation (Ib). Monocytes differentiate under initial inflammatory conditions to inflammatory macrophages (M1), which contribute to elimination of the initial trigger (injury, infection, etc.) together with neutrophils (Ic). Under active inflammation, hypoxia develops and supports further attraction of neutrophils. As a part of the trigger elimination process, neutrophils release their nucleic DNA and other cytoplasmic components in the process called NETosis, which contributes to pathogen elimination but also to further attraction of next waves of neutrophils (Id). The local inflammatory environment is sensed also by the peripheral nervous system (n. vagus) as a part of a neural circuit activated by cytokines, etc., known as the inflammatory reflex. Several factors, which initially contribute to the development of the inflammatory response, later contribute to resolution of inflammation as shown in the lower part of the scheme. Neutrophil microvesicles concentration-dependently blocks production of proinflammatory mediators in macrophages and NK cells probably with contribution of the macrophage-expressed phosphatidylserine receptor and further induces local production of anti-inflammatory TGF-β and IL-10 by dendritic cells and macrophages. Neutrophil microvesicles may also exhibit their anti-inflammatory function directly by their components such as phosphatidic acid (PA), docosahexaenoic acid (DHA) d5-17-HDHA, and d5-14-HDHA, which are biosynthetic pathway markers of resolvins and maresin,
AnxA1, S100A8, etc. PA exhibits inflammation resolving activities when hydrolysed by sPLA2 into lysophosphatidic acid (Ra). The nervous system contributes to resolution of inflammation by the inflammatory reflex consisting in the production of (1) noradrenalin, which acts upon T cells leading to production of immunosuppressive acetylcholine, and (2) Netrin-1, which together with acetylcholine contributes to changes in lipid mediator production from proinflammatory prostaglandins, thromboxanes, and leukotrienes to anti-inflammatory lipoxins, resolvins, maresins, protectins, etc. (Rb). Hypoxia, initially supporting the inflammation development, induces the blockage of neutrophils influx through Netrin-1 produced by endothelial cells in the later phases of inflammation and stimulates Th cells for extracellular release of anti-inflammatory-acting adenosine, which contribute to macrophage polarisation towards anti-inflammatory M2. Hypoxia is sensed through Hif-1α. (Rc). During resolution of inflammation, apoptotic cells exposing AnxA1 and PS are engulfed by efferocytosis (Rd), which is further stimulated by resolvins and lipoxins and induces polarisation of macrophages towards M2. Phagocytosis of apoptotic neutrophils induces egress of macrophages to draining lymph nodes (Re). Egress of cells from the inflammatory site is supported also by eosinophil-produced lipoxin A4. Another mechanism contributing to the resolution of inflammation consists in exaggerated NETosis leading to aggregation of NETs (AggNETs). Aggregated NETs degrade inflammatory cytokines, dismantle chemokine and cytokine gradients, and suppress further neutrophil recruitment (Rf). During resolution phases, the influx of neutrophils from the bloodstream is further inhibited by released AnxA1 a resolvins acting upon FPR2/ALX receptor (Rg). AggNETs = aggregated NETs; AnxA1 = annexin A1; d5-17-HDHA = 17-hydroxydocosahexaenoic acid; DC = dendritic cell; DHA = docosahexaenoic acid; FPR2/ALX = formyl peptide receptor 2; Hif-1α = hypoxia inducible factor 1; MPO = myeloperoxidase; NK = natural killer; PA = phosphatidic acid; PLA2 = secreted phospholipase A2; TGF = transforming growth factor.
Figure 4.
Fibroblasts, epithelia, and immune cells involvement in stromal address codes signals production in physiological and perpetuating inflammation. The recruitment of cells into a tissue occurs across vascular endothelium and is orchestrated by a set of cellular interactions involving capture receptors (selectins), activation molecules (chemokines), and adhesion receptors (integrins) designating endothelial address code. Fibroblasts directly affect the behaviour of infiltrating cells by providing retention, differentiation, and exit codes specified as a stromal address code. The development of an inflammatory response is associated with formation of lymph node-like structures with more or less structured resemblances to secondary lymph node, shown in the lower part of the scheme, but with the presence of activated macrophages, fibroblasts, and neighbouring pericytes and endothelial cells. During acute (physiologic) inflammation, the stromal address code differs from secondary lymph node by a spectrum of contributing cytokines and chemokines CXCL1-11, CCL2-5, CX3CL1, IL-1β, TNF-α, and IFN-γ. Pathologic inflammation, typical for chronic inflammation processes, is characterised by the appearance of some chemokines, cytokines, and growth factors such as CXCL12, CXCL13, CCL19, CCL21, IL-6, and VCAM-1, typically associated with secondary lymph node. Furthermore, pathological inflammatory conditions are perpetuated by IL-1β, IL-17, and TNF-α. Activated fibroblasts can be derived locally (by proliferation or trans-differentiation) and distally (from the bone marrow). Whether distally or locally derived fibroblasts predominate in different pathological...
inflammatory processes, and whether this depends on the presence or absence of epithelium in target organs remain to be elucidated. IL = interleukin; TNF-α = tumour necrosis factor.
**Table 1**

Cells contributing to inflammation and inflammation resolution.

| Cell         | General functions                  | Role in inflammation development                                                                 | Role in inflammation resolution                                                                 |
|--------------|------------------------------------|---------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Macrophages  | Phagocytosis                        | Inflammatory monocytes                                                                            | M2                                                                                                                                                           |
|              | Sensing DAMP                        | Tissue-resident MΦ                                                                                 | Efferocytosis                                                                                                                                             |
|              | Antigen presentation                | IL-1, TNF-α, chemokines, ω-6 FA metabolites                                                        | Egress to LN                                                                                                                                             |
|              |                                    |                                                                                                    | TGF-β, IL-10                                                                                                                                             |
|              |                                    |                                                                                                    | ω-6 AA to lipoxins switch                                                                                                                                  |
|              |                                    |                                                                                                    | ω-3 EPA and ω-3 DHA to resolvins, maresins, protectins                                                                                                    |
| Neutrophils  | Phagocytosis                        | ROS                                                                                               | AggNets cleavage of cytokines and chemokines                                                                                                              |
|              | NETosis                             | Microvesicles release                                                                             | Microvesicles release                                                                                                                                     |
|              |                                    | Apoptosis                                                                                         | Apoptosis                                                                                                                                                 |
|              |                                    | Extended longevity                                                                                | Egress to LN                                                                                                                                             |
|              |                                    |                                                                                                    | AnxA1                                                                                                                                                     |
| Eosinophils  | Phagocytosis                        | Release of toxic basic proteins and lipid mediators                                               | IL-4, IL-13 secretion                                                                                                                                     |
|              | Extracellular killing                | LTC4, PAF, TXA2, ECP                                                                              | Lipoxin A4 production                                                                                                                                   |
|              | Cytotoxic substances                |                                                                                                    |                                                                                                                                                            |
| Mast cells   | Secretin of vasoactive substances   | Sensing some DAMPs, PAMPs, secretion of heparin, histamine, chemokines ω-6 FA metabolites (prostaglandin-E2, thromboxane A2, leucotriene-B4) | ω-3 FA metabolites secretion                                                                                                                                |
| DC           | Sensing DAMP                        | Proinflammatory cytokines secretion                                                               | TGF-β, IL-10 secretion                                                                                                                                     |
|              | Antigen phagocytosis and presentation|                                                                                                  |                                                                                                                                                            |
| Fibroblasts  | Tissue support cytokine secretion   | Cytokine, chemokine, growth factors induce stromal address code                                  | Cytokine, chemokine, growth factors induce abnormal stromal address code                                                                               |
| Nervous system| Coordination                        | Inflammatory reflex abnormalities could enhance inflammation                                      | Inflammatory reflex, noradrenalin secretion                                                                                                               |
|              |                                    |                                                                                                    | Netrin-1 secretion                                                                                                                                         |
| B cells      | Antibody production                 | Immune complexes activating complement                                                            | Removal of antigen                                                                                                                                       |
| Endothelia   | Regulate transudation and exudation  | Adhesion and inflammatory cells transmigration support                                             | Hypoxia sensing Netrin-1 secretion                                                                                                                        |
|              |                                    |                                                                                                    | Acetylcholine inhibits release of TNF-α, IL-1β and IL-18                                                                                                  |
| CD4 T cells  | Cytokine secretion                  | Type 1 IFN                                                                                        | Sensing hypoxia                                                                                                                                              |
|              | proinflammatory/Anti-inflammatory   | IFN-γ                                                                                             | TGF-β, IL-10                                                                                                                                             |
|              | Regulatory function                 | TNF-α                                                                                             | Acetylcholine production                                                                                                                                   |
|              |                                    | IL-17                                                                                              | Adenosine release                                                                                                                                          |

Abbreviations: AA = arachidonic acid; AnxA1 = annexin A1; DAMP = danger-associated molecular patterns; DC = dendritic cell; DHA = docosahexaenoic acid; ECP = eosinophil cationic protein; EPA = eicosapentaenoic acid; FA = fatty acid; IL = interleukin; LTC4 = leucotriene C4; PAF = platelet-activating factor; ROS = reactive oxygen species; TGF = transforming growth factor; TNF = tumour necrosis factor; TXA2 = thromboxane A2.
Table 2

Detecting of danger, damage, and microenvironment stimuli by macrophage receptors.

| Trigger/stimulus                  | Source            | Sensor         | Sensor class | Cellular localisation | Response                           |
|----------------------------------|-------------------|----------------|--------------|-----------------------|------------------------------------|
| Lipopolysaccharide               | Bacteria          | TLR4/TLR4      | TLR          | Cell surface          | Type I IFN                         |
| Uropathogenic bacteria           |                   | TLR11/TLR11    | TLR          |                       | Proinflammatory cytokines          |
| Triacylated lipopeptides         |                   | TLR2/TLR1      | TLR          |                       |                                    |
| Diacylated lipopeptides          |                   | TLR2/TLR6      | TLR          |                       |                                    |
| dsRNA                            | Viruses           | TLR3/TLR3      | TLR          | Endosome              |                                    |
| CpG DNA                          | Bacteria          | TLR9/TLR9      | TLR          | Endosome              |                                    |
| ssRNA                            | Viruses           | TLR7/TLR8      | TLR          | Endosome              |                                    |
| DNAs                             | Any               | AM2            | Inflammasome | Cytoplasm             | IL-1β, IL-18                       |
|                                  |                   | Polymerase III/RIG-I | RLR     | Cytoplasm             | Type I IFN                         |
|                                  |                   | HMGB1          | RAGE/TLR7    | Cell surface          | Proinflammatory cytokines          |
|                                  |                   | cGAS           | cGAMP synthase| Cytoplasm             | Type I IFN                         |
|                                  |                   |                |              |                       | Proinflammatory cytokines          |
|                                  |                   |                |              |                       | Type I IFN                         |
| β-Glucan                         | Bacteria Fungi    | TLR            | TLR          | Cell surface          | Type I IFN                         |
|                                  |                   | Decin-1, -2    | CLR          |                       | Proinflammatory cytokines          |
| mannosae                         | Bacteria Fungi    | MR             | CLR          |                       | Phagocytosis                        |
|                                  |                   | Langerin       |              |                       |                                    |
| Long dsRNA                       | Viruses           | MDA5           | RLR          | Cytoplasm             | Type I IFN                         |
|                                  |                   |                |              |                       | Proinflammatory cytokines          |
| Muramyl dipeptide                | Bacteria          | NOD2           | NLR          | Cytoplasm             | Type I IFN                         |
| g-D-Glutamyl-meso-diaminopimelic acid | Bacteria          | NOD1           | NLR          | Cytoplasm             | Proinflammatory cytokines          |
|                                  |                   |                |              |                       | IL-1β, IL-18                       |
| Flagellin                        | Bacteria          | TLR5/TLR5      | TLR          | Cell surface          | Type I IFN                         |
|                                  |                   |                |              |                       | Proinflammatory cytokines          |
| Pore-forming toxins              | Bacteria Dead cells | NLRC4       | Inflammasome | Cytoplasm             | IL-1β, IL-18, HMGB1, IL-1α          |
|                                  |                   | NLRP3          | Inflammasome | Cytoplasm             | "Find me" signal                   |
|                                  |                   | P2Y2           | Inflammasome | Cytoplasm             | Type I IFN                         |
|                                  |                   | RAGE           | GPCR         | Cell surface          | Proinflammatory cytokines          |
|                                  |                   |                |              |                       | Type I IFN                         |
|                                  |                   |                |              |                       | Proinflammatory cytokines          |
|                                  |                   |                |              | "Find me" signal      |                                    |
|                                  |                   |                |              |                       | "Eat me" signal                    |
| Uric acid crystal                |                  | NLRP3          | Inflammasome | Cytoplasm             | IL-1β, IL-18, HMGB1, IL-1α          |
| Phosphatidyl serine + MFG-E8 or Gas6 |                  | Tim-4         | Type I membrane proteins | Cell surface | "Eat me" signal                    |
|                                  |                   | BAI1           |              |                       |                                    |
|                                  |                   | Stablin-2 (HARE)|             |                       |                                    |
| Trigger/stimulus | Source | Sensor | Sensor class | Cellular localisation | Response |
|-----------------|--------|--------|--------------|----------------------|----------|
| Phosphatidyl serine + Gas6 or protein S |          | Tyro3 | TAM          | Cell surface         | “Eat me” signal |
|                 |        | Axl    |             |                      |          |
|                 |        | Mer    |             |                      |          |
| LPC             |        | G2A    | GPCR         | Cell surface         | “Find me” signal |
| S1P             |        | S1P-R  |             |                      |          |
| Hypoxia         | Tissue microenvironment | HIF-1α | HIF-1α       | Cell surface         | Infection resolution |
| pH              |        | GPR65  | GPCR         |                      |          |
| Heat            |        | TRPV2  | Cation channel |                    | Phagocytosis, chemotaxis |
| Osmolarity      |        | NLRP3  | Inflammasome | Cytoplasm            | IL-1β, IL-18, HMGB1, IL-1α |
|                 |        | NLRP4  |             |                      |          |

Proinflammatory cytokines such as IL-6, IL-12, TNF-α, GM-CSF, IL-8. Type I IFN such as IFN-α and IFN-β1.

BAI1 = brain-specific angiogenesis inhibitor 1; CLR = C-type lectin receptor; dsRNA = double-stranded RNA; Gas6 = growth arrest-specific 6; GM-CSF = granulocyte–macrophage colony-stimulating factor; GPCR = G protein coupled receptor; HARE = hyaluronic acid receptor for endocytosis; HMG1 = high mobility group box 1; IFN = interferon; LPC = lysophosphatidylcholine; MFG-E8 = milk fat globule EGF factor 8; MR = mannose receptor; NLR = NOD-like receptor; NLRP3 = NAIP, CIITA, HET-E, and TP1 (NACHT)-, Leucine-rich repeat (LRR)- and Pyrin domains (PYD)-containing protein; P2Y2 = purinergic receptor; pH = potential of hydrogen; RAGE = receptor of advanced glycation end products; RLR = RIG-I-like receptor; S1P = sphingosine-1-phosphate; ssRNA = single-stranded RNA; TAM = Tyro3, Axl, and Mer; Tim-4 = T cell immunoglobulin and mucin-domain-containing molecule 4; TLR = Toll-like receptor; TRPV2 = transient receptor potential vanilloid 2.
**Table 3**

Proresolving mediators and their function.

| Proresolving mediator | Type | Source | Receptor | Sensing cells and function |
|-----------------------|------|--------|----------|---------------------------|
| Resolvins             | ω-3 EPA and ω-3 DHA metabolite | M2 Precursors in neutrophil microvesicles | GPCRs | Block neutrophil transmigration, enhance monocyte recruitment, stimulate efferocytosis, induce apoptosis |
| Annexin A1            | Glucocorticoid regulated protein | Released from neutrophil vesicles, present in microvesicles, apoptotic bodies | FPR2/ALX | Blocks leucocyte transmigration, stimulates efferocytosis, induces apoptosis |
| Protectins            | ω-3 EPA and ω-3 DHA metabolite | M2 Eosinophils Perivascular adipose tissue | GPCRs | Block neutrophil transmigration, protect neurons, enhance monocyte recruitment, enhance efferocytosis. |
| Lipoxins              | ω-6 AA metabolite | Neutrophils, leucocytes | GPCRs | Block neutrophil transmigration, induce egress of leucocytes to lymph node, stimulate efferocytosis |
| Maresins              | ω-3 DHA metabolite | M2 Precursors in neutrophil Microvesicles | GPCRs | Block neutrophil transmigration, induce M1 to M2 polarisation, block ROS production and NF-κB pathway, enhance monocyte recruitment and efferocytosis, tissue regeneration. |
| Chemerin-derived peptides | Generated by proteolytic cleavage of preproteins | Liver, adipose tissue, lung, skin, pancreas, adrenal gland | ChemR23 | Block neutrophil transmigration, induce chemotaxis of MDM, DC, and NK cells, adipogenesis, osteoblastogenesis, angiogenesis |
| Extracellular adenosine | Nucleotide (purine) | T cells, extracellular concentrations rise during metabolic stress, ischaemia, hypoxia, inflammation and trauma | A1, A2A, A2B, A3 adenosine receptors | Macrophage polarization towards M2, inhibition of Th1 cytokine production, effector molecule of Treg cells |
| Netrin-1              | Neuronal guiding protein | n. vagus Endothelia in response to hypoxia | A2B adenosine receptor | Blocks neutrophil transmigration, suppresses hypoxia-elicited inflammation, promotes lipid proresolving metabolites |
| Proresolving mediator | Type        | Source   | Receptor | Sensing cells and function                       |
|-----------------------|-------------|----------|----------|--------------------------------------------------|
| Acetylcholine         | Neurotransmitter | T cells | AChR     | Blocks proinflammatory mediators from MΦ           |

AChR = acetylcholine receptor; DC = dendritic cell; FA = fatty acid; FPR2/ALX = formyl peptide receptor 2; GPCR = G protein coupled receptor; Maresin = macrophage mediator in resolving inflammation; MDM = monocyte-derived macrophage; NF-κB = nuclear factor kappa B; ROS = reactive oxygen species; ω–3 DHA = ω–3 docosahexaenoic acid; ω–3 EPA = ω–3 eicosapentaenoic acid; ω–6 AA = ω–6 arachidonic acid.