Chapter 5

Neutrophils in Rheumatoid Arthritis: A Target for Discovering New Therapies Based on Natural Products

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Additional information is available at the end of the chapter

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Abstract

Rheumatoid arthritis (RA) is a systemic autoimmune disorder with an important inflammatory component in joints. Neutrophils are the most abundant leukocytes in inflamed joints, and play an essential role in the initiation and progression of RA. Neutrophil effector mechanisms include the release of proinflammatory cytokines, reactive oxygen and nitrogen species (ROS and RNS), and granules containing degradative enzymes, which can cause further damage to the tissue and amplify the neutrophil response. Therefore, the modulation of neutrophil migration and functions is a potential target for pharmacological intervention in arthritis. The pharmacologic treatment options for RA are diverse. The current treatments are mostly symptomatic and have side effects, high costs, and an increased risk of malignancies. Because of these limitations, there is a growing interest in the use of natural products as therapies or adjunct therapies. Herbal products have attracted considerable interest over the past decade because of their multiple beneficial effects such as their antioxidant, anti-inflammatory, antiproliferative, and immunomodulatory properties. This chapter focuses on the role of neutrophils in the pathogenesis of arthritis and the action of substances from natural products as putative antirheumatic therapies.

Keywords: neutrophils, rheumatoid arthritis, herbal products, polyphenols, flavonoids, tetranortriterpenoids, inflammation

1. Introduction

Arthritis is an inflammatory joint disorder that can cause edema, pain, and loss of function. The most common types of arthritis are osteoarthritis, gout, and rheumatoid arthritis [1, 2]. Rheumatoid arthritis is a systemic, autoimmune disorder with an important inflammatory

component in which genetic and environmental risk factors contribute to disease development. Its prevalence in the world population is between 0.3 and 1%, and it affects three times more women than men [3, 4].

The pathophysiology of RA is complex and appears to be initiated when the adaptive immune system (cellular or humoral) recognizes self-joint antigens as non-self, which triggers a variety of distinct inflammatory effector mechanisms, including the recruitment of leukocytes [5–8].

RA is characterized by intense inflammatory processes and joint damage that are mediated by the influx of immune system cells to the synovial space such as neutrophils, macrophages, and lymphocytes [1, 2]. A critical factor that contributes to tissue damage is the excessive production of inflammatory mediators by resident and/or infiltrated cells. Among the primary mediators involved in joint damage are free radicals, enzymes that degrade the matrix, and pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β, as well as chemokines such as CXCL-8, lipid mediators, such as leukotriene B_4 (LTB_4) [9, 10], and endothelin (ET) [11, 12]. Inflamed synovial tissue is invasive and called pannus, which can be formed by synovial cell proliferation, angiogenesis, and the accumulation of macrophages, lymphocytes, and neutrophils [13].

Neutrophils are crucial cells that have significant roles in diverse inflammatory diseases, including acute, chronic, autoimmune, infectious, and non-infectious conditions [14]. The most well-known effector function of neutrophils is their role in innate immunity. However, recent studies have identified neutrophils as active cells during adaptive immunity, facilitating the recruitment and activation of antigen-presenting cells or directly interacting with T cells. Neutrophils are the most abundant leukocytes in inflamed joints, and the importance of these cells in the initiation and progression of human RA as well as in murine models has been demonstrated [15–18]. Therefore, neutrophils play an essential role in joint inflammation, and the modulation of neutrophil functions is considered a potential target for pharmacological intervention in arthritis [19–21].

The pharmacologic treatment options for arthritis are diverse. The current treatments are mostly symptomatic and include non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, disease-modifying antirheumatic drugs (DMARDs), and biologic therapies. High costs and an increased risk of malignancies limit the use of these agents, in addition to the potential side effects that all therapies possess. Plant-derived products, such as polyphenols, sesquiterpenes, flavonoids, and tetranortriterpenoids, which are herbal metabolites with anti-inflammatory activity, may provide new therapeutic agents and cost-effective treatments [22, 23]. This chapter focuses on the role of neutrophils in the pathogenesis of arthritis and the action of substances from natural products as putative antirheumatic therapies.

2. Role of neutrophils in rheumatoid arthritis

2.1. Neutrophil trafficking from blood to the synovial cavity

Neutrophil recruitment is an important stage in the inflammatory development process, including autoimmune diseases such as RA. Among the circulating cells, neutrophils are the first ones to reach the synovium and are the most abundant cells in the synovial fluid [24]. In this section,

we discuss the cascade of events that culminates in neutrophil entry into inflamed joints. The leukocyte recruitment cascade involves the following commonly recognized steps: capture, rolling, firm adhesion, and finally transendothelial migration.

Neutrophil release from the bone marrow to the circulating blood occurs immediately after the first signal of inflammation, serving to increase the number of neutrophils available for recruitment into the tissue in response to inflammation [25]. The mobilization of neutrophils from the bone marrow is orchestrated by the hematopoietic cytokine granulocyte colony-stimulating factor (G-CSF). G-CSF mobilizes neutrophils indirectly by shifting the balance between CXCR4 and CXCR2 ligands [26]. In response to the release of inflammatory mediators such as TNF-α and IL-17, the adjacent vascular endothelium becomes activated. Cell surface proteins of the selectin family termed E- and P-selectin and their ligands (L-selectin) mediate this initial neutrophil capture. Neutrophil rolling through the endothelium facilitates their contact with chemotactic factors that promotes neutrophil activation [27]. Chemokines (CXCR-1 or 2 ligands, such as IL-8), the C5a fragment of the complement system, and leukotriene B_4 (LTB_4) are responsible for neutrophil mobilization to the synovial fluid [28–30].

Firm adhesion is mediated by interactions between β_2 integrins (LFA-1, CD11a/CD18, and MAC-1, CD11b/CD18) and their ligand (ICAM-1). Integrins are usually in an inactive state on neutrophil and become activated after the triggering of G protein-coupled receptors such as chemokine receptors [31]. The binding of integrins to their ligands activates signaling pathways in neutrophils stabilizing adhesion and initiating cell motility [32, 33]. This signaling also regulates actin polymerization, which controls the direction of neutrophil movement [34, 35]. The final stage in the adhesion cascade is the ultimate migration of the neutrophil from the vasculature into the inflamed tissue. Passage through the endothelial cell layer occurs both paracellularly (between endothelial cells) and by a transcellular route (over the endothelial cell). Paracellular migration of neutrophils is mediated by binding to endothelial proteins that target neutrophils to intercellular junctions and facilitate their passage through them. To reach the inflamed joint, neutrophils must pass over the basal membrane, which occurs through the degradation of extracellular matrix molecules by proteases stored inside the cells, such as matrix metalloproteinases (MMPs) and serine proteases [14].

In inflammatory foci, neutrophils find immune complexes on the synovium that bind to Fcγ receptors on the neutrophil membrane, triggering their degranulation and reactive oxygen species (ROS) production [36]. In RA pathology, oxidative stress is a result of inadequate ROS release by neutrophils [37]. Oxygen radicals cause DNA damage and oxidation of lipids, proteins, and lipoproteins and may be involved in immunoglobulin mutations that lead to rheumatoid factor (RF) formation [38, 39]. Moreover, proteins from neutrophil degranulation are found at high concentrations in the RA synovial fluid and could be responsible for cartilage and tissue damage, activation of cytokines and soluble receptors, inhibition of chondrocyte proliferation and activation of synoviocytes proliferation and invasion [40–43]. In addition, activated neutrophils also generate chemoattractants (such as IL-8 and LTB_4) that promote further neutrophil recruitment and amplify the inflammatory response (see **Figure 1**).

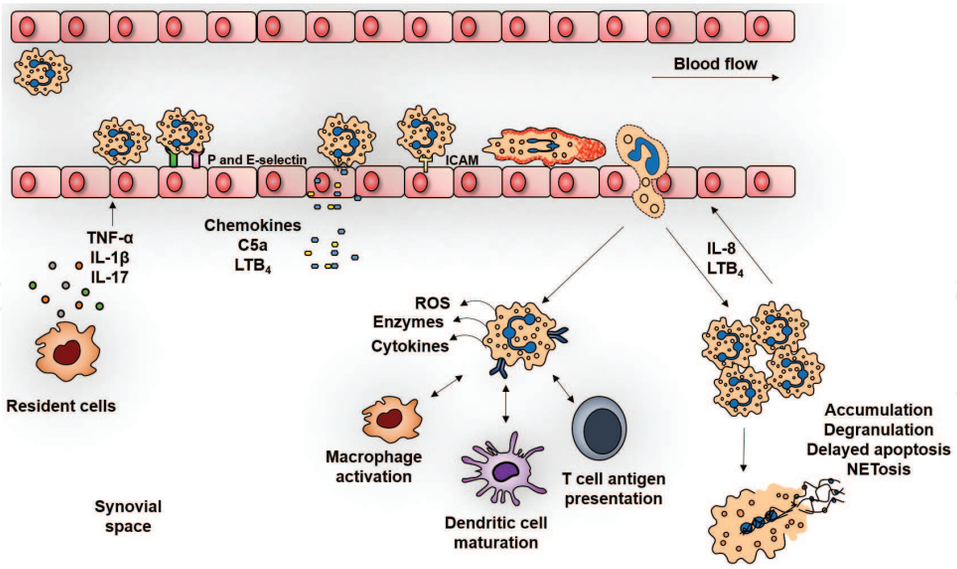

Figure 1. Overview of the role of neutrophils in arthritis. Neutrophils leave blood vessels after chemotactic signals from inflamed tissues that promote the firm adhesion of neutrophils to endothelial cells mediated by adhesion molecules, which induce neutrophil activation and actin filament formation followed by transendothelial migration toward the inflammatory foci. Immune complexes and proinflammatory molecules activate neutrophils, which then produce ROS and release enzymes responsible for cartilage destruction. Activated neutrophils communicate with other cells of the immune system through the secretion of cytokines and chemokines and by antigen presentation in conjunction with MHC class II. Neutrophils can undergo a special form of cell death called NETosis. This results in the release of a complex of nuclear and granule molecules called NETs contributing to tissue damage. Activated neutrophils also generate chemoattractants (such as IL-8 and LTB_4), forming a positive-feedback loop that promotes further neutrophil recruitment and amplifies the acute inflammatory response. Finally, effective neutrophil apoptosis is required for the resolution of inflammation. However, delayed neutrophil apoptosis occurs in the inflamed joint, which results in persistent inflammation and tissue damage due to the continued release of ROS, granule enzymes, and cytokines.

2.2. Neutrophil action in rheumatoid arthritis

Neutrophils are key cells in articular inflammation that are abundant in the synovial fluid and pannus of patients with active RA [44], a typical knee joint may have 2×10^9 cells, of which 90% are neutrophils [24]. These cells are mobilized to synovial tissue by chemoattractant mediators, such as CXCL1, CXCL2, endothelin (ET)-1, and leukotriene B_4, a process in which resident macrophages play a central role [11, 45, 46].

For many years, the major contribution of neutrophils to the pathology of RA was thought to be their cytotoxic potential, since neutrophils participate in the pathogenesis of arthritis by promoting the inflammatory process and cartilage degradation, as well as bone resorption. However, neutrophils are now recognized to have an active role in orchestrating the progression of inflammation through regulating the functions of other immune cells [47, 48], and current research has shown that these cells are involved in RA onset [49, 50].

In the synovial cavity, activated neutrophils exhibit an increased expression of plasma membrane receptors such as major histocompatibility complex (MHC) class II molecules and present antigens to T lymphocytes, an immune function that they share with macrophages and dendritic cells (DCs) [51]. In addition, the interaction of neutrophils with other cells induces the secretion of MMP-8 and MMP-9, and a repertoire of cytokines (IL-1β, IL-12, IL-18, IL-23,

and TNF-α) and chemokines (CCL-2, CCL-4, CCL-5, and CXCL-8), including TNF ligand superfamily member (RANKL) [52, 53] and TNFSF13B (also known as BLyS or BAFF) [54], which are implicated in the activation of osteoclasts and B lymphocytes, respectively, regulate the function of other immune cells [48, 55–57].

Neutrophils from patients with RA are functionally very different from those isolated from healthy individuals. RA blood neutrophils are already primed for ROS production [58] and striking differences in gene and protein expression exist between peripheral blood neutrophils from patients with RA and their healthy counterparts [18], including higher levels of membrane-expressed TNF and myeloblastin (also known as PR-3 or cANCA antigen) in RA [59].

In RA patients, neutrophils can be activated by immune complexes, such as RF or anti-citrullinated protein antibodies (ACPAs), both within the synovial fluid and deposited on the articular cartilage surface [60]. These complexes engage Fcγ receptors and thereby trigger neutrophil activation, which release ROS and RNS [61, 62], collagenases, gelatinases, neutrophil myeloperoxidase (MPO), elastase, and cathepsin G into the synovial fluid and joints [14, 55, 56, 63] due to frustrated phagocytosis [60].

2.2.1. Pain in rheumatoid arthritis and neutrophils

One of the most prevalent symptoms of RA is the increase in sensitivity to joint pain (hyperalgesia), which causes movement limitations. Despite its clinical relevance, strategies for the treatment of arthralgia remain limited. In animal models, hyperalgesia (inflammatory pain) is defined as hypernociception (a decreased nociceptive threshold) [64]. It is broadly accepted that articular hypernociception results mainly from the direct and indirect effects of inflammatory mediators on the sensitization (increased excitability) of primary nociceptive fibers that innervate the inflamed joints [65–67]. Prostaglandins and sympathetic amines are the key mediators of this process. Furthermore, other mediators, such as the cytokines TNF-α, IL-1β, IL-6, and IL-17 play a crucial role in the pathogenesis of arthritis, increasing the recruitment of neutrophils into the joint and driving the enhanced production of chemokines and degradative enzymes [68–70]. In addition, endothelin-1 (ET-1), acting directly or indirectly, also sensitizes primary nociceptive neurons [71–74].

During the inflammatory process, the migrating neutrophils participate in the cascade of events leading to mechanical hypernociception, by mediating the release of hyperalgesic molecules (such as MPO, MMPs, hypochlorite, superoxide anion, and PGE_2) capable of activating nociceptive neurons and causing pain [17, 75–78].

Indeed, decreased inflammation and joint destruction have been directly correlated with reduced neutrophil influx into the joints, as observed in mouse models by means of antibody blockade or the gene deletion of chemoattractant receptors such as CXCR1, CXCR2, and BLT1 (LTB_4 receptor) [15, 79]. Therefore, the blockade of neutrophil migration could be a target in the development of new analgesic drugs [77].

2.2.2. Citrullinated autoantigens and NETs in rheumatoid arthritis

Citrullination is the natural posttranslational conversion of arginine to citrulline mediated by peptidyl arginine deiminases (PADs), enzymes present in macrophages, dendritic cells, and

neutrophils. Experimental evidence indicates that citrullination is involved in the breakdown of immune tolerance and may generate neoantigens (neoAgs) that become additional targets during epitope spreading [80]. Citrullinated residues stimulate the production of anti-citrullinated protein antibodies (ACPAs) in predisposed individuals. It has been observed that ACPAs can be present for several years before any clinical signs of arthritis appear [81–83]. A substantial increase in the number and titer of many antibodies against posttranslationally modified proteins is also seen shortly before the onset of arthritis. Citrullinated Ags have increased immunogenicity and arthritogenicity, and their presence in arthritic joints correlates with disease severity [80, 84–86].

Osteoclasts are dependent on citrullinating enzymes for their normal maturation and display citrullinated antigens on their cell surface in a non-inflamed state. In humans, the binding of ACPAs to osteoclasts in the bone compartment induces IL-8 secretion. In turn, IL-8 sensitizes and/or activates sensory neurons by binding to CXC chemokine receptor (CXCR) 1 and CXCR2 on peripheral nociceptors [87–90], producing IL 8 dependent joint pain that is associated with ACPA-mediated bone loss.

IL-8 release contributes to the chemoattraction of neutrophils [49], which play critical roles in initiating and maintaining joint-inflammatory processes that have been described in experimental arthritis [36, 91]. However, the exact roles that neutrophils play in the posttranslational modification of proteins and disease initiation and progression in RA remain unclear. Recent evidence suggests that, among the various mechanisms by which neutrophils cause tissue damage and promote autoimmunity, aberrant formation of neutrophil extracellular traps (NETs) could play important roles in the pathogenesis of RA [50].

NETs are released during a process of cellular death named NETosis. NETosis occurs with neutrophils upon contact with bacteria, fungi [92], or under several inflammatory stimuli. This process is associated with changes in the morphology of the cells, which eventually lead to cell death with extrusion of NETs [93, 94]. This process requires calcium mobilization, reactive oxygen species (ROS) produced by NADPH oxidase, neutrophil chromatin decondensation mediated by neutrophil elastase (NE) and myeloperoxidase (MPO), and chromatin modification via the citrullination of histones by peptidyl arginine deiminase 4 (PAD4) [95–99]. NETs are a network of extracellular fibers, which contain nuclear compounds as DNA and histones and that are covered with antimicrobial enzymes and granular components, such as MPO, NE, cathepsin G, and other microbicidal peptides [93, 94]. In the extracellular environment, NET fibers entrap microorganisms, and their enzymes and granular substances reach locally high concentrations and are thus able to cleave virulence factors and kill microorganisms [95, 100, 101].

Although NETs play a key role in the defense against pathogens, they may cause undesirable effects to the host, which has increased the interest in the role of neutrophils and NETs in autoimmunity. Augmented NET formation was first described in preeclampsia and ANCA-associated vasculitis and followed by the description in a series of autoimmune conditions, including psoriasis, systemic lupus erythematosus (SLE), antiphospholipid antibody syndrome (APS), and RA [50, 100, 102–105]. Neutrophil extracellular traps are an obvious source of nuclear material. Among these are a range of cytoplasmic and extracellular citrullinated antigens, well-established

targets of the ACPAs found in RA [50, 100]. The protein contents of NETs not only serve as targets for autoantibody and immune complex formation but also induce further NETosis, resulting in a harmful positive-feedback loop. These factors form an inflammatory microenvironment that may trigger a strong autoimmune response in individuals with the corresponding susceptibility [106, 107]. Pro-inflammatory cytokines, such as TNF-α and IL-17, as well as autoantibodies stimulate the formation of NETs and affect their protein composition [50]. Additionally, NETs have been shown to stimulate autoimmunity via the production of interferons and activation of the complement cascade. Interferons activate both the innate and adaptive immune systems, inducing a Th1 immune response and stimulating B cells toward the generation of autoantibodies [108]. The deposition of NETs observed in various inflammatory pathologies is associated with the circulating cell-free DNA (cfDNA) levels in biological fluids, such as plasma and serum, from patients [100, 101, 109]. Therefore, circulatory cfDNA could eventually be utilized as a marker of NETs in these pathologies, while the determination of the DNA levels might facilitate the monitoring of disease activity and assessment of the effectiveness of a selected therapeutic strategy.

Neutrophils have been traditionally viewed as short-lived cells that die at sites of inflammation; however, some evidence suggests that they can prolong their life span upon specific stimuli and transmigrate away from inflammatory loci [48, 110, 111]. Conditions within the synovial joint, such as hypoxia [112] and the presence of antiapoptotic cytokines (including TNF, granulocyte-macrophage colony-stimulating factor (GM CSF), and IL 8) [113, 114], can increase neutrophil survival for up to several days [115, 116], which contributes to enhanced tissue damage.

As described above, neutrophils play an essential role on innate and adaptive immunity in RA physiopathology, contributing to tissue lesions in RA, and therefore represent a promising pharmacological target in RA. Pharmacological strategies that inhibit or reduce neutrophil mobilization or activation could be successful in RA treatment.

3. Neutrophils as therapeutic targets

Animal models have been extensively used in studies of RA pathogenesis. Despite the inherent limitations of all animal models, several rodent models have greatly contributed to the overall knowledge of important processes/mediators in the generation of inflammation, cartilage destruction, and bone resorption. In addition, the pharmaceutical industry has used these models for testing potential anti-arthritic agents, leading to important advances in therapeutic interventions for this destructive disease [117]. Such models include collagen-induced arthritis, collagen antibody-induced arthritis, zymosan-induced arthritis, the methylated BSA model, and genetically manipulated or spontaneous arthritis models such as the TNF-α-transgenic mouse, K/BxN mouse, and Skg mouse [118]. Many of these models show that neutrophils are the first immune cells to enter the arthritic joint, and that early measures of joint inflammation correlate with neutrophil infiltration [45, 119, 120]. In this section, we highlight pharmacological approaches targeting neutrophil recruitment and activity, which present a therapeutic benefit to patients with RA.

The current treatments available to RA patients include glucocorticoids, non-steroidal anti-inflammatory drugs, and disease-modifying antirheumatic drugs. Only disease-modifying agents—and to some extent glucocorticoids—can impede or halt the inflammatory and destructive disease processes [121]. With a more complete understanding of the immune-inflammatory events that occur in the pathogenesis of RA, scientists have developed therapeutic strategies that include monoclonal antibodies and receptor constructs, which target specific soluble or cell-surface molecules of interest. Biological agents such as monoclonal antibodies and recombinant proteins that target TNF-α, CD20, CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), and the IL-1 receptor as well as therapies based on the blockade of T-cell and B-cell functions have shown efficacy in controlling the physical signs and pain associated with RA [122, 123].

Many interventions used to treat RA exert inhibitory effects on neutrophil responses in inflammation. However, non-steroid anti-inflammatory drugs (NSAIDS), DMARDs, and biologics do not specifically target neutrophil function [124].

Most NSAIDs inhibit the action of the cyclo-oxygenase-1 and -2 (COX-1 and -2) enzymes, which metabolize arachidonic acid into inflammatory mediators of the prostaglandin family. NSAIDs have been shown to inhibit neutrophil adherence, decrease degranulation and oxidant production, inhibit neutrophil elastase activity, and induce neutrophil apoptosis [125–127]. Corticosteroids induce anti-inflammatory signals by several mechanisms; a major one may be to reduce the expression of cytokine-induced genes. They enter all cells and bind to the cytoplasmic steroid receptor, and then this complex translocates to the nucleus where it is recognized by specific DNA sequences. The major effect of binding to DNA is the suppression of transcription by opposing the activation of the transcription factors AP-1 and NF-κB [128]. Corticosteroids have been shown to inhibit neutrophil degranulation and ROS production, decrease production of inflammatory mediators, and prevent neutrophil adhesion and migration into RA joints [44, 129–131]. The most widely used DMARD in clinic settings is methotrexate, a compound that blocks folic acid metabolism. Its benefits in RA include the stimulation of neutrophil apoptosis [116], inhibition of the NF-κB pathway [132], and reduced adhesion molecule expression and LTB_4 production [133], consequently decreasing neutrophil recruitment and ROS production [134].

Anti-TNF-α therapies are also widely used for the treatment of RA patients. TNF primes the neutrophil respiratory burst, upregulates the expression of adhesion molecules, cytokines and chemokines, and at high local concentrations can stimulate ROS production in adherent neutrophils [135–138]. Three different TNF inhibitors are available for RA patients who fail to respond adequately to standard DMARD therapy. Infliximab and adalimumab are monoclonal antibodies against TNF, whereas etanercept is a TNFRII fusion protein. All three drugs sequester soluble TNF [139]. Reports regarding the direct effect of anti-TNF agents on neutrophils have been published, and these drugs have been shown to decrease the mobilization of neutrophils from the peripheral blood to inflamed joints [140], decrease *ex vivo* neutrophil ROS production [20], and reduce neutrophil chemotactic and adhesive properties [141].

Tocilizumab, a monoclonal antibody that blocks the soluble and tissue-expressed IL-6 receptor, is also proving to be a highly effective biologic agent in RA treatment [142]. Neutrophils are a major source of soluble IL-6 receptors, which they shed in large quantities when activated, and their accumulation in high numbers within the synovial joint could contribute significantly to IL-6 signaling within the synovium through trans-signaling [143]. *In vivo* therapeutic blockade of IL-6 with tocilizumab induces transient neutropenia caused by apoptosis or phagocytosis of apoptotic neutrophils but does not impair antibacterial neutrophil functions [144].

Despite the clinical efficacy of these therapies, many patients do not exhibit significant responses or discontinue treatment because of adverse effects. In addition, the limited availability of biological agents in developing countries, the need for parenteral administration of these products, and the high cost restrict access to such therapies for many RA patients worldwide, and this promotes a continuous search for new therapeutic targets and the development of new drugs [145]. Due to these limitations, interest has grown in the use of alternative treatments and herbal therapies for arthritis patients [146, 147] (**Table 1**).

Therapy	Effect on neutrophil response	Reference
Non-steroidal anti-inflammatory drugs (NSAIDS)	Inhibit neutrophil adherence, decrease neutrophil degranulation and ROS production, inhibit neutrophil elastase activity, and induce neutrophil apoptosis	[125–127]
Corticosteroids	Inhibit neutrophil degranulation and ROS production, decrease the production of inflammatory mediators, and prevent neutrophil adhesion and migration into RA joints	[44, 129–131]
Disease-modifying antirheumatic drugs (DMARDs)	Stimulate neutrophil apoptosis, inhibit the NF-κB pathway, and reduce adhesion molecule expression, LTB_4 production, neutrophil recruitment, and ROS production	[116, 132–134]
TNF-α inhibitors	Decrease neutrophil mobilization from the peripheral blood to inflamed joints and reduce *ex vivo* neutrophil ROS production and neutrophil chemotactic and adhesive properties	[20, 140, 141]
IL-6 inhibitor	Induce transient neutropenia caused by apoptosis or phagocytosis of apoptotic neutrophils but not impair antibacterial neutrophil functions	[144]

Table 1. Current therapeutic targets for arthritis and their effect on neutrophils.

4. Plant-derived molecules as emerging therapies for arthritis

Current arthritis treatments result in unwanted side effects and tend to be expensive, and natural products devoid of such disadvantages offer a novel opportunity. The use of natural products represents a promising alternative to treat rheumatic diseases, in particular by acting as therapeutic adjuvants to reduce the daily doses of conventional drugs that RA patients administer [148–150]. In this section, we highlight future perspectives in the treatment of RA with natural compounds, mainly herbal compounds, to minimize the harmful effects of the over-activation of neutrophils.

Decreased inflammation and joint destruction have been directly correlated with reduced neutrophil influx into the joints, as observed in mouse models by means of antibody blockade or the gene deletion of chemoattractant receptors such as CXCR1, CXCR2, and BLT1 (LTB_4 receptor) [15, 79]. The prospect of new drugs obtained from herbal products (or from structures of herbal products) plays a compelling role in drug discovery and development [151].

As previously mentioned, pharmacologic treatment options for arthritis are diverse and present several side effects. Furthermore, the high costs and increased risk of malignancies limit the use of such agents. Because of these limitations, there is a growing interest in the use of natural products as therapies or adjunct therapies [22]. Plant-derived products such as polyphenols, sesquiterpenes, flavonoids, and tetranortriterpenoids, which are herbal metabolites, are considered to have potential activity to block inflammation, and they may provide new therapeutic agents and cost-effective treatments [22, 23]. These natural products have attracted considerable interest over the past decade because of their multiple beneficial effects, such as their antioxidant, anti-inflammatory, antiproliferative, and immunomodulatory properties. In this section, we discuss the plant-derived products that have been most studied in RA experimental models and/or clinical trials (**Table 2**).

4.1. Quercetin

Quercetin (**Figure 2a**) is the major dietary flavonol found in fruits, vegetables, and beverages, such as tea and red wine [152]. Several epidemiological and experimental studies support the antioxidant, anti-inflammatory, antiangiogenic, antiproliferative, and proapoptotic effects of this molecule [153–155]. Preclinical studies on primary cells and animal models, as

Compound	Chemical class	Arthritis experimental model	Reference
Quercetin	Flavonoid	Adjuvant-induced arthritis	[156]
Methyl gallate	Polyphenol	Zymosan-induced arthritis	[171]
Gedunin	Tetranortriterpenoid	Zymosan-induced arthritis	[176]
Epigallocatechin gallate	Polyphenol	Collagen-induced arthritis	[179]
Curcumin	Polyphenol	Collagen-induced arthritis	[191]

Table 2. Herbal products that exhibit anti-arthritic potential in animal models.

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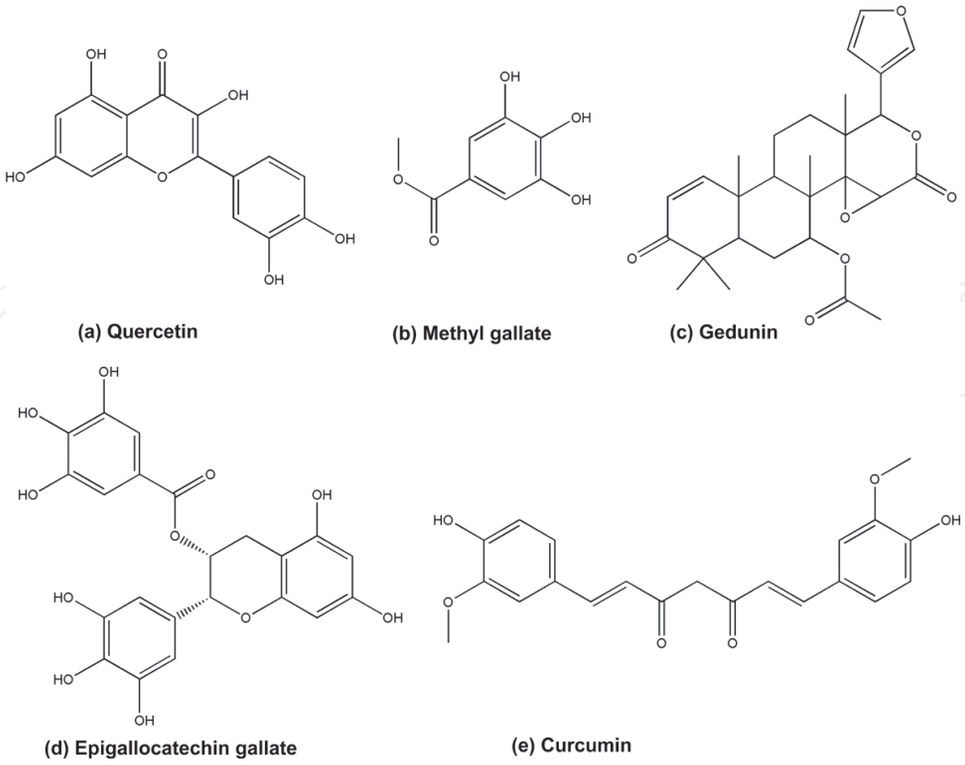

Figure 2. Chemical structure of (a) quercetin, (b) methyl gallate, (c) gedunin, (d) epigallocatechin gallate, and (e) curcumin.

well as clinical studies, suggest an inhibitory action of quercetin in RA. Quercetin has been reported to lower the levels of IL-1β, C-reactive protein, and monocyte chemotactic protein-1 (MCP-1), and restore plasma antioxidant capacity. In addition, quercetin increased the expression of hemeoxygenase-1 in the joints of arthritic rats. Finally, quercetin inhibited the twofold increase in NF-κB activity observed in joints after arthritis induction [156].

There are divergent data on the effect of quercetin in neutrophils. For instance, *in vitro*, quercetin inhibited myeloperoxidase activity [157] but had no effect on lipopolysaccharide-induced neutrophil surface expression of the adhesion molecules L-selectin (CD62L) and β2 integrin (CD11b/Mac1), [158] which are related to rolling and firm adhesion, respectively [159]. In paw edema induced by carrageen, quercetin did not inhibit the increase in myeloperoxidase, which is used as a marker of neutrophil recruitment [160]. Therefore, it seems unlikely that quercetin would inhibit neutrophil recruitment [158]. On the other hand, quercetin inhibits the fMLP-induced increase in intracellular calcium, [158] which is necessary for actin polymerization and consequently neutrophil migration [159]. In addition, *in vitro*, quercetin blocked human neutrophil mobilization through the inhibition of the cellular signaling responsible for actin polymerization in association with the down-regulation of adhesion molecules [161], indicating that treatment with this flavonoid is a conceivable approach to control excessive neutrophil recruitment during inflammation and to prevent neutrophil-mediated tissue lesions [162] (**Table 3**).

4.2. *Schinus terebinthifolius* and methyl gallate

S. terebinthifolius Raddi (Anacardiaceae) is a native plant from South America. It has been used in folk medicine as teas, infusions, or tinctures, as an anti-inflammatory, febrifuge, analgesic,

Compound	Molecular targets/mechanisms	Reference
Quercetin	Inhibits IL-1β, C-reactive protein, and MCP-1 levels. Restores plasma antioxidant capacity, increases HO-1 expression, and inhibits NF-κB activity in joints Inhibits myeloperoxidase activity in neutrophils and blocks neutrophil mobilization	[156, 157, 161]
Methyl gallate	Reduces edema formation, total leukocyte accumulation, neutrophil migration and IL-6, TNF-α, CXCL-1, IL-1β, $LTB_{4,}$ and PGE_2 production in zymosan-induced arthritis. Impairs neutrophil chemotaxis and adhesion	[171]
Gedunin	Attenuates zymosan-induced articular edema, neutrophil migration, hypernociception, and the production of IL-6, TNF-α, $LTB_{4,}$ and PGE_2 and prevents increases in lipid bodies. Decreases neutrophil shape changes, chemotaxis, and lipid body formation	[176]
Epigallocatechin gallate	Ameliorates the severity of arthritis and regulates the expression of cytokines, chemokines, MMPs, ROS, NO, COX-2, and PGE_2. Affects neutrophil functionality and inhibits IL-8 and MIP-3α expression	[179–184, 186–189]
Curcumin	Suppresses collagen-induced arthritis by reducing cellular infiltration, synovial hyperplasia, cartilage destruction, and bone erosion. Blocks neutrophil recruitment	[191, 193]

Table 3. Major molecular targets and anti-arthritic mechanisms of herbal products.

and depurative agent and to treat urogenital system illnesses [163]. Scientific reports demonstrated that *S. terebinthifolius* extracts and fractions are rich in polyphenols and display antioxidant, antibacterial, and antiallergic properties in different experimental models [164–166]. The HPLH chromatograms of hydroalcoholic extracts from *S. terebinthifolius* leaves (ST-70) reveal that methyl gallate (MG, **Figure 2b**) is one of the major polyphenol components of the ST-70 extract [167]. Methyl gallate has been extensively studied because of its antioxidant, antitumor, and antimicrobial activities [168–170]. Pharmacological studies have shown that ST-70 and MG also have an anti-inflammatory effect and may have potential activity against arthritis. Pretreatment with ST-70 or MG markedly reduced knee-joint thickness, total leukocyte (mainly neutrophil) infiltration, and reduced the production of inflammatory mediators associated with arthritis such as CXCL-1/KC, IL-6, TNF-α, IL-1β, $LTB_{4,}$ and PGE_2. ST-70 and MG also inhibited murine neutrophil chemotaxis induced by CXCL-1/KC *in vitro*, and

MG impaired the adhesion of these cells to TNF-α-primed endothelial cells [167, 171]. These results provide some evidence that MG inhibits neutrophil activation and adhesion molecules expression and consequently prevents the neutrophil entry into inflammatory sites (**Table 3**).

Moreover, unlike potassium diclofenac, the long-term oral administration of ST-70 does not induce lethality or gastric damage in mice, which suggests that ST-70 could be used to treat inflammatory conditions such as arthritis with less toxicity [167].

4.3. *Carapa guianensis* and gedunin

C. guianensis Aublet is a member of the Meliaceae family that is widely used in folk medicine in Brazil and other countries surrounding the Amazon rainforest [172]. Anti-inflammatory and analgesic activities are among the most remarkable properties attributed by ethnopharmacological research to the oil extracted from *C. guianensis* seeds, mainly for rheumatic pain and arthritis [172, 173]. *C. guianensis* oil and six different tetranortriterpenoids (TNTP) isolated from the oil were able to significantly inhibit zymosan-induced knee joint edema formation and protein extravasation. TNTP pretreatment inhibited the increase in total leukocyte and neutrophil numbers in the synovial fluid. TNTP also impaired the production of TNF-α, IL-1β, and CXCL-8/IL-8, and significantly inhibited the expression of the NF-κB p65 subunit [174].

Gedunin (**Figure 2c**) is a natural tetranortriterpenoid isolated from vegetal species of the Meliaceae family and is known to inhibit the stress-induced chaperone heat shock protein (Hsp) 90 [175]. Mouse pretreatment and posttreatment with gedunin impaired zymosan-induced edema formation and total leukocyte influx mainly due to the inhibition of neutrophil migration and reduced articular hypernociception. Gedunin also reduced the *in situ* expression of preproET-1 mRNA and IL-6, TNF-α, LTB_4 and PGE_2 production and prevented increases in the number of lipid bodies in synovial leukocytes [176]. Lipid bodies are important sites for the synthesis and storage of lipid mediators and they increase in number during inflammatory responses [177]. In neutrophils, gedunin impaired ET-1-induced shape changes, blocked ET-1- and LTB_4-induced chemotaxis, decreased ET-1-induced lipid body formation and impaired neutrophil adhesion to TNF-α-primed endothelial cells [176]. The combined *in vitro* and *in vivo* effects of gedunin reveal its potential as an anti-arthritic candidate, especially its direct effect on key cells involved in articular inflammation such as neutrophils (**Table 3**).

4.4. Epigallocatechin gallate

Epigallocatechin gallate (EGCG, **Figure 2d**) is one of the main components of green tea [178]. It has antioxidative, anti-inflammatory, antitumor, and chemopreventive properties. The potential disease-modifying effects of green tea on arthritis have been reported; for example, in a mouse model of RA, the induction and severity of arthritis was ameliorated by the prophylactic administration of green tea polyphenols [179]. Subsequent studies suggested that EGCG possesses remarkable potential to prevent chronic diseases like OA and RA [180–184]. The anti-inflammatory and anti-arthritic effects of EGCG are supported by *in vitro* and *in vivo* data indicating that EGCG can regulate the expression of cytokines, chemokines, MMPs,

ROS, nitric oxide (NO), COX-2, and PGE_2 in cell types relevant to the pathogenesis of RA [179–184]. In *in vivo* studies, EGCG was found to inhibit inflammation in mouse models by affecting the functioning of T cells and neutrophils [185, 186]. IL-8 is the most powerful chemo-attractant for neutrophils in the target tissue. EGCG is a very effective inhibitor of IL-1β and of TNF-α-induced IL-8 and macrophage-inflammatory protein-3α (MIP-3α) expression in different cell types [187–189]. These *in vitro* and *in vivo* observations indicated the efficacy of EGCG and demonstrate that it can modulate multiple signal transduction pathways in a fashion that suppresses the expression of inflammatory mediators that play a role in the pathogenesis of arthritis (**Table 3**).

4.5. Curcumin

Curcumin (**Figure 2e**) is a yellow-colored polyphenol found in the rhizome of turmeric. It has antioxidant, anti-inflammatory, antiapoptotic, and anticarcinogenic properties [190]. Oral administration of curcumin suppressed type II collagen-induced arthritis (CIA) in mice by reducing cellular infiltration, synovial hyperplasia, cartilage destruction, and bone erosion. Moreover, the production of MMP-1 and MMP-3 was inhibited by curcumin in CIA and in TNF-α-stimulated RA fibroblast-like synoviocytes (RA-FLS) and chondrocytes [191].

In vitro, it has been reported that curcumin decreases IL-1β-induced expression of the pro-inflammatory cytokine IL-6 and vascular endothelial growth factor (VEGF) in RA-FLS [192]. In addition, curcumin blocks neutrophil recruitment through the inhibition of cellular signaling responsible for actin polymerization in association with the down-regulation of adhesion molecules [193]. It has also been shown to induce apoptosis of RA-FLS (which are resistant to apoptosis) by increasing the expression of the proapoptotic protein Bax and down-regulating the expression of the antiapoptotic protein Bcl-2 [190]. Some molecular mechanisms related to curcumin have been identified. In a human synovial fibroblast cell line (MH7A) stimulated with IL-1β, curcumin blocked the activation of the NF-κB pathway and induced deactivation of the ERK-1/2 pathway [192]. In addition, this polyphenol inhibited activating phosphorylation of protein kinase Cδ (PKCδ) in CIA, RA-FLS, and chondrocytes. Curcumin also suppressed JNK and c-Jun activation in those cells [191].

In a clinical trial with RA patients, curcumin reduced reported pain, tenderness, and swelling of joints [194]. A curcumin-based medicine, Meriva®, demonstrated efficacy in clinical trials with patients with osteoarthritis by reducing reported pain [195]. In another clinical trial, treatment with Meriva® reduced stiffness and physical signs of RA (treadmill test) along with IL-1, IL-6, and VCAM-1 production [196] (**Table 3**).

5. Conclusion

In RA, neutrophils are key cells that are recognized to play an active role in orchestrating the progress of inflammation, through the release of pro-inflammatory cytokines, ROS, RNS, and NETs, which potentially affect the activities of both neutrophils and other cell types, such as resident mononuclear cells and chondrocytes. In addition, neutrophils participate in the

cascade of events leading to mechanical hypernociception. Therefore, neutrophils participate in the pathogenesis of arthritis by promoting the inflammatory process, degradation of cartilage, and bone resorption. The modulation of neutrophil migration and functions in RA can be considered a potential target for pharmacological intervention in arthritis. The pharmacologic treatment options for arthritis are diverse. High costs and an increased risk of malignancies limit the use of these agents, in addition to the potential for side effects that all therapies possess. Nevertheless, herbal metabolites with anti-inflammatory activity and inhibitory action in neutrophils may provide new therapeutic agents and cost-effective treatments.

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