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## Review Article

# Innate immunity drives pathogenesis of rheumatoid arthritis

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## ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune disease affecting ~1% of the general population. This disease is characterized by persistent articular inflammation and joint damage driven by the proliferating synovial tissue fibroblasts as well as neutrophil, monocyte and lymphocyte trafficking into the synovium. The factors leading to RA pathogenesis remain poorly elucidated although genetic and environmental factors have been proposed to be the main contributors to RA. The majority of the early studies focused on the role of lymphocytes and adaptive immune responses in RA. However, in the past two decades, emerging studies showed that the innate immune system plays a critical role in the onset and progression of RA pathogenesis. Various innate immune cells including monocytes, macrophages and dendritic cells are involved in inflammatory responses seen in RA patients as well as in driving the activation of the adaptive immune system, which plays a major role in the later stages of the disease. Here we focus the discussion on the role of different innate immune cells and components in initiation and progression of RA. New therapeutic approaches targeting different inflammatory pathways and innate immune cells will be highlighted here. Recent emergence and the significant roles of innate lymphoid cells and inflammasomes will be also discussed.

Rheumatoid arthritis (RA) is a common autoimmune disorder that affects 1% of the population worldwide [1]. The incidence of RA is higher in females compared to males with an incidence ratio of 2:1 and 3:1, respectively [2]. The disease is characterized by persistent articular inflammation and joint

damage driven by the proliferating synovial tissue fibroblasts as well as T and B lymphocytes, neutrophils and monocytes trafficking into the synovium [3]. Inflammation also causes synovium to hypertrophy, resulting in an abnormal tissue called pannus, which invades and destroys local articular

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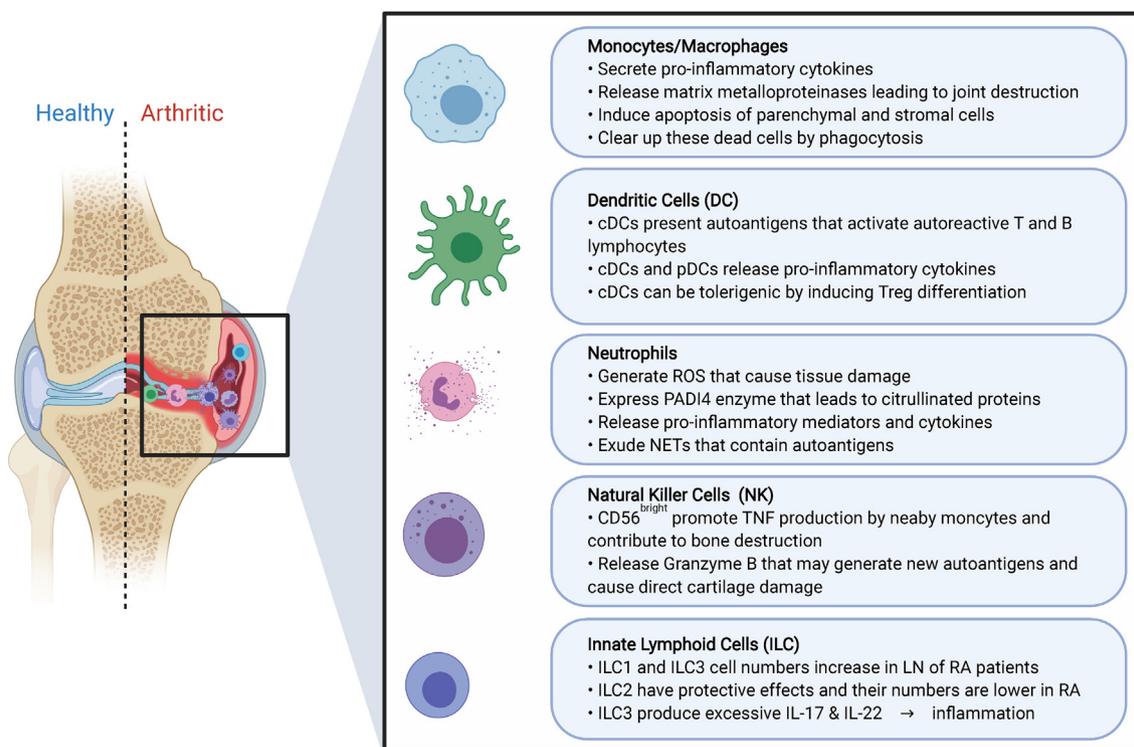
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**Fig. 1 Contribution of various innate immune cells to RA pathogenesis.** Monocytes, macrophages, dendritic cells, neutrophils, natural killer cells and innate lymphoid cells play a key role in the early stages as well as in the progression of rheumatoid arthritis. Figure was created with [BioRender.com](https://www.biorender.com).

structures. Cells in the RA pannus express pro-inflammatory cytokines, chemokines and matrix metalloproteinases that contribute to progressive cartilage and bone destruction [3,4]. The etiopathology of RA is not fully understood; however, several genetic and environmental factors have been implicated [5]. It was established decades ago that certain HLA-DRB1 alleles are associated with susceptibility to RA [6]. Large genome-wide association studies (GWAS) have now identified over 100 loci involved in RA pathogenesis [7]. Phosphatase protein tyrosine phosphatase non-receptor type 22 (PTPN22) shows the second strongest association with RA and encodes lymphoid tyrosine phosphatase, or Lyp, an important negative regulator of T-cell receptor signaling. Other relevant non-HLA gene single nucleotide polymorphisms (SNPs) associated with RA include CTLA4, tumor necrosis factor receptor family (TNFR) associated factor TRAF1, transcription factor STAT4, chemokine receptor CCR6, interferon regulatory factor 5 (IRF5), and PADI4, an enzyme that mediates the citrullination of proteins (reviewed in Ref. [8]). All these genes are important for immune regulation. PTPN22, CTLA4 and STAT4 are involved in T-cell stimulation, activation, and functional differentiation, while others like TRAF1 and IRF5 are implicated in nuclear factor- $\kappa$ B (NF- $\kappa$ B)-dependent signaling [8,9].

Two clinically useful diagnostic markers have been identified for RA: anti-cyclic citrullinated peptide antibodies (ACPA) and rheumatoid factor (RF) [10]. RF is a high-affinity autoantibody against the Fc portion of immunoglobulin, while ACPA are antibodies to autoantigens modified by citrullination through deamination of arginine to citrulline.

ACPA are present in about two-thirds of all RA patients but occur in less than 2% of healthy individuals [11].

In addition to genetic factors, environmental factors play a significant role in the development of RA (reviewed in Ref. [12]). Smoking and other forms of lung stress, such as exposure to silica, may trigger the development of ACPA seropositive RA. A smoking history has an especially strong influence on the risk of developing RA in HLA-DRB1 patients [12]. Other environmental and lifestyle-related factors including exposure to infectious agents (e.g. Epstein-Barr virus, cytomegalovirus, *Porphyromonas gingivalis*), and birthweight have been linked with rheumatoid arthritis (reviewed in Refs. [10,13]).

Activation of innate immunity in the synovium by TLR agonists or Fc receptor engagement occurs early in RA and serves as a key pathogenic mechanism that leads to inflammation. Cells of the innate immune system such as monocytes, macrophages and dendritic cells (DCs) have a critical place in innate immunity through their function as phagocytes, antigen-presenting cells and cytokine producers and play a significant role in initiating and perpetuating the disease [4,14,15]. Synovial dendritic cells activated by TLR ligands can migrate to lymph nodes where primed T cells can be biased towards the  $T_H1$  phenotype, and through chemokine receptors like CCR5, home to inflamed synovial tissue [16,17]. Production of cytokines and expression of adhesion molecules after activation of innate immunity in the joint then permits the continued ingress of immune cells. Many of the cytokines and chemokines produced by innate cells are directly implicated in many of the immune processes that are associated with the pathogenesis of rheumatoid arthritis [4]. (see Figs. 1 and 2).

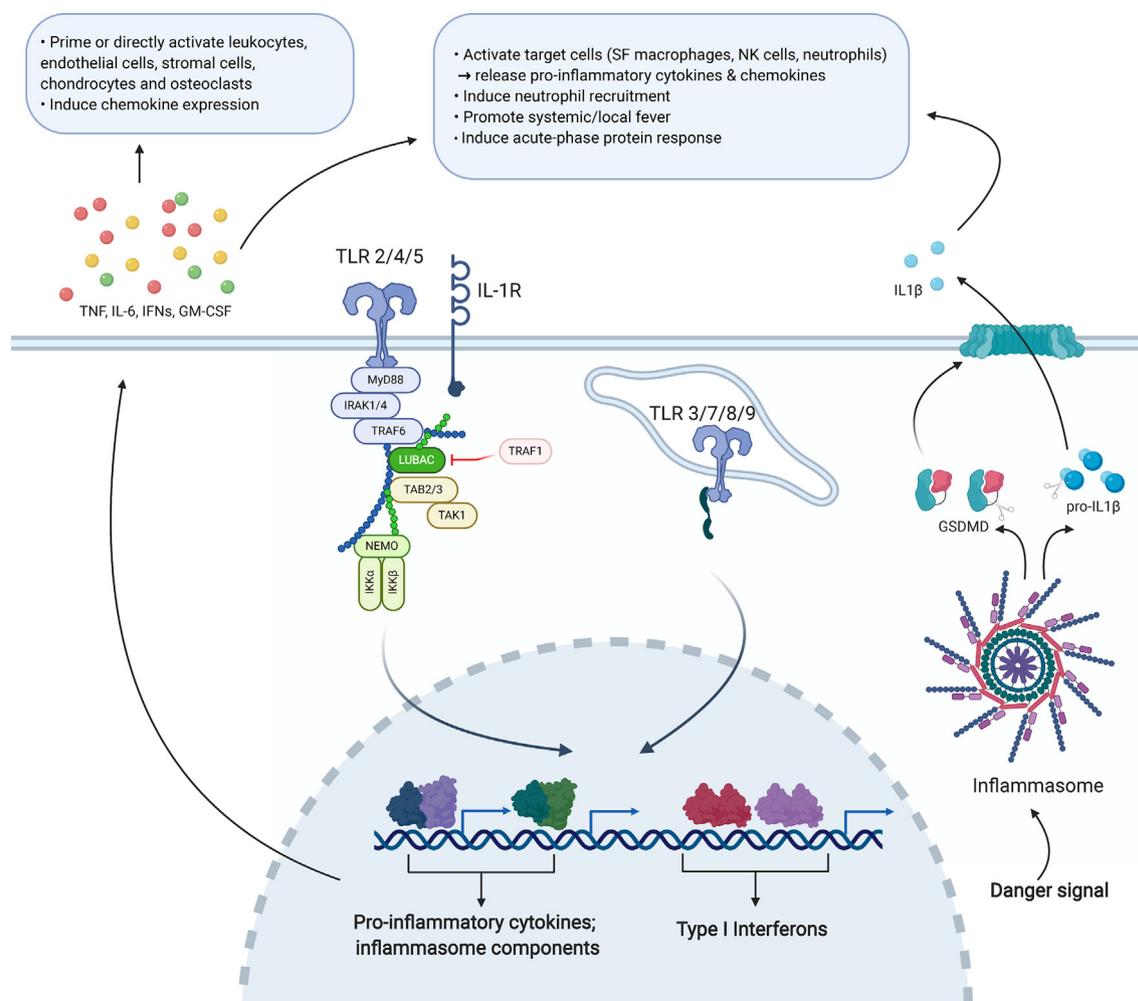


Fig. 2 **Innate immune signaling during in RA.** Upon ligand binding, Toll-like receptors (TLR) and Interleukin-1 receptor (IL-1R) activate a series of intracellular signaling events that lead to the activation of distinct transcription factors, which culminate in the induction of gene transcription and subsequent secretion of various pro-inflammatory cytokines (e.g. TNF, IL-6, type I interferons (IFN)) and growth factors (e.g. GM-CSF). However, one of these cytokines, IL-1 $\beta$ , cannot be secreted unless it gets processed first by caspase-1. Caspase-1 itself is a zymogen and its activity requires the assembly of a molecular complex, called the inflammasome, which is activated when a danger signal has been sensed. Caspase-1 also cleaves gasdermin D (GSDMD) into two fragments (N and C terminus). The N-terminal fragment of GSDMD then oligomerize and form a pore in the plasma membrane, which allows for the release of active IL-1 $\beta$  and other cellular components. All these cytokines then act locally or systemically to promote an inflammatory status in the synovium and activate/attract various immune cells that initiate and contribute to RA pathogenesis. Figure was created with [BioRender.com](https://www.biorender.com)

This review summarizes the current state of our knowledge as well as recent advances regarding the role of innate immune cells and signaling in RA pathogenesis. We will also provide some insight into how therapeutic strategies that target innate immunity can be utilized to treat RA.

### The role of monocytes and macrophages in RA pathogenesis

Macrophages play a central role in initiating and driving the pathogenesis of rheumatoid arthritis [18–20]. These cells are major sources of cytokines, chemokines and degradative enzymes that drive joint inflammation and ultimately lead to the destruction of cartilage and bone. In addition, macrophages

and their products are thought to be involved in synovial angiogenesis, which, in turn, plays a key role in pathogenesis of RA (reviewed in Refs. [21]). The number of synovial tissue macrophages is clinically important as it is the most reliable marker for assessing disease severity and response to therapy as the number of myeloid cells correlates with RA synovial inflammation, radiographic progression and disease activity [22,23].

Recruited, short-lived populations of mononuclear phagocytes that patrol different tissues are characterized by high surface expression of Ly6C, CCR2 and CD11b, and are significantly increased during disease course [24]. However, long-lived, self-renewing tissue-resident macrophages that originate primarily from embryonic progenitors can be found in the joint as well [24,25]. Circulating monocytes infiltrate from

the blood into the inflamed RA joint where they differentiate into macrophages. Macrophages, whether recruited or tissue resident, are known for their phenotypic heterogeneity and plasticity. They can be polarized to become classically activate, “M1-like” macrophages, which are considered to be pro-inflammatory, or alternatively activated, “M2-like” macrophages, which possess anti-inflammatory properties and can initiate tissue repair [26]. Generally, In RA tissues, M1-like macrophages overexpress MHC class II molecules, which indicate their activation and promotion of inflammation and tissue damage (Kinne et al., 2000). They secrete a variety of pro-inflammatory cytokines in the joints of patients affected by RA, such as TNF, IL-1 $\beta$ , IL-8, IL-15, IL-18 and macrophage migration inhibitory factor (MIF) (reviewed in (McInnes et al., 2016)). In addition, macrophages are responsible for inflammation damage through the release of matrix metalloproteinases (Blom et al., 2007). On the other hand, M2-like macrophages release anti-inflammatory cytokines, such as IL-4, IL-10, PGE2 and TGF- $\beta$ , which initiate tissue repair and remodeling and contribute to vasculogenesis [27]. Moreover, IL-4 and TGF- $\beta$  may induce macrophages to favor matrix deposition [28]. However, it is important to note that in the context of a complicated disease like RA, macrophages exist on a wide spectrum between the M1- and M2-like phenotypes [19,27]. Future studies that employ single-cell RNA seq analysis should be able to accurately identify the various synovial macrophage subsets in RA patients, which may lead to novel therapeutic approaches that target specific macrophage subsets in RA.

To summarize, the pathogenic roles of monocytes and macrophages in RA are mainly due to the production of pro-inflammatory cytokines, chemokines, growth factors and free radicals, and the release of matrix metalloproteinases that lead to joint inflammation and destruction.

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### The complex roles of dendritic cells in RA

The involvement of dendritic cells (DCs) in tolerance and autoimmunity is complex and bidirectional. Indeed, DCs might promote tolerance through multiple mechanisms, including through the generation and maintenance of T<sub>Reg</sub> cells, as well as through the induction of T cell unresponsiveness [29]. Conversely, the antigen presentation capacity of DCs might promote the priming and/or the effector differentiation of self-reactive T cells. In inflamed RA synovial tissue, most antigen presenting cells (APCs) are fully differentiated DCs expressing high levels of class I and II MHC and T cell costimulatory molecules [30,31]. Flow cytometry and histologic analyses of DC subsets have shown a trend toward a reduced number of circulating DCs in RA patients associated with a concomitant increase in the inflamed tissue [31]. DC subsets differ considerably in localization, cytokine secretion, and immunological function. There are two main DC subsets involved in RA pathogenesis: conventional DCs (cDCs), also known as classical DCs, and plasmacytoid DCs (pDCs). cDCs can be broadly subdivided into two subsets, cDC1 and cDC2, which are specialized in presenting endogenous and exogenous antigen on both MHC-I and II, to CD8 and CD4 T cells, respectively [32]. Conversely, pDCs are found circulating in the

blood and in peripheral organs and are uniquely able to rapidly produce large amounts of type I interferons upon viral infection [32]. The accumulation of cDCs in autoimmune sites can be a consequence of the increased expression of chemokine receptors or their specific ligands in the tissue. For example, cDCs express the CCL20 receptor, CCR6, which mediates the attraction of DC and Th17 cells to the tissues and allows mature cDCs to accumulate in the perivascular region of RA patients' synovium [33]. Alternatively, defective migration to the draining lymph nodes from the inflamed tissue may be the cause of accumulation. It is believed that a local maturation process mediates the sequestration of DCs in the leukocyte aggregates in the inflamed tissue of RA patients [34]. In addition, cell-free RA synovial fluid facilitated DC maturation from myeloid progenitors, providing direct evidence that the inflamed RA joint environment instructs DC growth [30,31]. Mature cDCs can then polarize naïve T lymphocytes into Th1, Th2, T<sub>Reg</sub>, or T<sub>H</sub>17 through the secretion of different sets of cytokines [35].

The accumulation of danger signals in the inflamed tissue stimulates and drives the cDCs to immunogenic or tolerogenic profiles. The release of cytokines that prime an improper autoantigen presentation leads to dysregulated autoreactive T and B lymphocytes that contribute to the physiopathology of autoimmune disorders. Both cDCs and pDCs contribute to RA pathogenesis and disease progression by secreting a large number of cytokines, including TNF, IL-1, IL-12, IL-6, Interferons (IFNs) as well as differentiation factors, including macrophage colony stimulating factor (M-CSF) and fibroblast growth factor (FGF) [14]. Mature cDCs that produce high amounts of IL-12 and IL-23 have been reported in the infiltrates of synovial tissues of RA patients, suggesting that these cells have a role in the polarization of pathogenic T lymphocytes [31]. Inflammatory DCs, which are recruited to sites of inflammation or infection, induce the secretion of IL-17 in naïve CD4 T cells through the secretion of TGF $\beta$ , IL-1 $\beta$ , IL-6, and IL-23 [31,36]. Activated cDCs also produce high levels of B lymphocyte activation and survival factors, such as BAFF and APRIL, which have a key role in B lymphocyte differentiation and antibody production (reviewed in Ref. [37]). On the other hand, cDCs can play a tolerogenic role by controlling T<sub>Reg</sub> differentiation [14]. In mouse models of RA, injection of fully mature DCs loaded with collagen prevents collagen-induced arthritis (CIA) after the induction of a T<sub>H</sub>2 shift [38]. In addition, immature DCs can expand and activate a novel regulatory population of CD49b<sup>+</sup> T cells, with high immunosuppressive potential able to mediate protection against a systemic autoimmune disease [39].

In summary DC-driven events have the ability to either induce tolerance or autoimmunity depending on the various cues they receive in the joint microenvironment. The phenotypic and functional plasticity of DCs highlight the complex and dichotomous role they might play in pathogenesis of RA.

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### The role of neutrophils in RA

Neutrophils are the first cells to reach the synovium and the most abundant leukocytes in inflamed joints [40]. The

importance of these cells in the initiation and progression of RA in patients as well as in murine models has been well documented. Neutrophils bind immune complexes on the synovium via their Fc $\gamma$  receptors on the neutrophil membrane, triggering their degranulation and reactive oxygen species (ROS) production [40,41]. In RA pathology, this enhanced ROS generation by neutrophils at the site of inflammation causes endothelial dysfunction and tissue injury. Oxygen radicals cause DNA damage and oxidation of lipids, proteins, and lipoproteins and may be involved in immunoglobulin mutations that lead to formation of autoantibodies [42]. Neutrophils also express the PADI4 enzyme responsible for the citrullination of arginine [43], and PADI4 deletion led to reduced disease severity as well as lower levels of autoantibodies and inflammatory cytokines in CIA mouse model [44]. Normally, these cells have a short lifespan and undergo apoptosis after 6–18 h in circulation. However, a defect in neutrophil clearance causes apoptotic neutrophils to undergo secondary necrosis [45]. The ingestion of this debris by macrophages then induces production of pro-inflammatory cytokines, consequently amplifying inflammation. In patients with early RA, synovial neutrophils show significantly decreased levels of apoptosis compared to patients with persistent forms of arthritis [46]. A number of stimuli, including IL-8, TNF and GM-CSF can activate neutrophils [47]. Activated neutrophils have been shown to secrete immune mediators including IL-1, IL-6, IL-12, TGF- $\beta$ , TNF, oncostatin M and BlyS, triggering positive regulatory feedbacks, which lead to acute and persistent inflammation [40]. Neutrophil extracellular traps (NETs), which consist of chromatin and contents of neutrophil granules, are typically released by neutrophils upon interaction with a pathogen. In RA, citrullinated histones released in NETs can be recognized by ACPAs and thereby serve as autoantigens [48]. Furthermore, synovial neutrophils of RA patients have a higher propensity to form NETs when induced with LPS or with certain ACPAs [49].

To diminish the effects of neutrophils in RA pathogenesis, current pharmacological therapies targeting neutrophils in RA are aimed at minimizing its inflammatory status in the synovial fluid. There are a number of different agents used to treat RA including non-steroidal anti-inflammatory drugs (NSAIDs), and of disease-modifying anti-rheumatic drugs (DMARDs), anti-TNF and anti-IL6 blocker [40]. TNF has pleiotropic effects on inflammation and neutrophil functions, such as priming the neutrophil respiratory burst, increasing the expression levels of other cytokines, chemokines and adhesion molecules, and stimulating ROS production. Blocking TNF activity, therefore, has the effect of blocking downstream function of neutrophil activation [50]. Several studies have demonstrated that NETs and their components are elevated in RA patients and can therefore be used as biomarkers and potential therapeutic targets [49]. Indeed, a recent report showed that inhibiting NETs by a therapeutic anti-citrullinated protein antibody (tACPA) leads to enhanced clearance of NETs by macrophages, which then attenuated tissue damage in the joints of a CIA mouse model [51]. Neutrophils play an integral role in RA and our ability to control its function will greatly diminish its 'destructive' features within the synovial space.

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## The role of NK cells in RA

Through their cytolytic capacity and generation of cytokines and chemokines, natural killer (NK) cells are a type of innate lymphoid cells (ILCs; see below) that play a critical role in tumour surveillance and early host defense against viruses [52]. A subset of NK cells accumulates in the inflamed RA synovial membrane and contributes to bone destruction. These cells are CD56<sup>bright</sup> and can promote TNF production by CD14<sup>+</sup> monocytes in a contact-dependent manner when activated with IL-12, IL-15, or IL-18 [53]. Other studies have shown increased expression of granzyme positive NK cells in early RA synovial fluid (Sf) compared with osteoarthritis [54]. Granzyme B plays a role in promoting autoimmunity, generating new epitopes, and inducing direct cartilage damage. As such, high serum levels of granzyme B have been shown to act as an independent predictor of early erosion in RF-positive individuals [55].

Recent studies began exploring differences in NK cell numbers, function and subsets between healthy donors and RA patients. Lin et al. characterized function and phenotypes of NK cells from peripheral blood of RA patients [56]. They showed that NK cell percentage in PBMCs of RA patients is higher than that of controls. This may be due to the higher serum levels of IL-15 they observed in RA patients. They also found decreased expression of Nkp46 and CD62L, but increased CD158b and CD158e expression on NK cells obtained from RA patients versus controls [56]. Another study identified differentially expressed genes in NK cells obtained from RA patients versus healthy controls. NK cells isolated from patients with RA expressed higher mRNA levels of CD56, CXCL16, PECAM-1, ITGB7, BTK, TLR10, and IL-1 $\beta$ , but lower levels of CCL2, CCR4, RELA and IBTK [57]. Yamin et al. showed that subpopulations of NK cells can vary depending on the severity of RA [58]. Activated synovial fluid (Sf)-NK cells in patients with advanced RA constituted around 25% of all lymphocytes, a much higher proportion than those in patients with milder non-deformative disease [58]. Together, these studies demonstrate that certain NK cell populations and genes can be used as biomarkers for RA progression or diagnosis.

Overall, NK cells play an important role in RA pathogenesis and current investigations deciphering specific subtypes of these cells along with their function in relation to RA manifestation may reveal novel therapy targets.

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## The role of innate lymphoid cells (ILCs) in RA

Innate lymphoid cells (ILCs) play an important role in inflammatory diseases including Crohn's disease, colorectal cancer, psoriasis and arthritis [59,60]. These cells function as a bridge between the innate and adaptive immune system and are characterized by the absence of recombination activating gene (RAG)-dependent rearranged antigen-specific receptors [59]. ILCs are mainly found at barrier surfaces in the body where they quickly respond to environmental stress signals, and play a role in tissue remodelling, protection against pathogens and tissue homeostasis [59,60]. There are three

different groups of innate lymphocytes (ILCs): ILC1, ILC2, ILC3 [59]. NK cells, discussed above, belong to the first group. Abnormal activation of ILCs (other than NKs) have been shown to be the contributing factor to RA pathogenesis. So far, there has been a limited number of studies supporting a role for other ILCs in RA pathogenesis. A recent report examined lymph node (LN) biopsy specimens from patients in the earliest phases of RA. No difference was found in the frequency of total ILCs, but patients with RA had greater numbers of ILC1s and ILC3s in their LNs than the healthy controls, and patients at risk of developing rheumatoid arthritis had higher levels of ILC1. The findings indicate that prior to the development and during the earliest phases of RA, ILC distribution in LNs changes from a homeostatic profile toward a more inflammatory one [61]. A more recent study, however, demonstrated that ILC3 CCR6<sup>+</sup> cells contribute to RA inflammation via excessive IL-17 and IL-22 production. Samples taken from a CIA mouse model (compared to control mice) along with samples taken from the synovium of RA patients (compared to healthy controls) indicated increased levels of chemokine ligand 20 (CCL20), IL-17A, and IL-22 [62]. The significance of IL-17 in the synovium of RA and juvenile idiopathic arthritis patients emphasizes a greater role for IL-17 in the overall manifestation of RA [63]. In contrast to ILC1 and ILC3, ILC2 levels decrease in the synovium of RA patients, while their number is higher in the joints/circulation when RA patients are in remission. Further investigation showed that ILC2 control T<sub>Reg</sub> activity. In mice, in the absence of ILC2 proliferation, T<sub>Reg</sub> were inactive resulting in enhanced inflammation and bone erosion. ILC2-induced T<sub>Reg</sub> activity led to resolution of inflammation and bone protection [64]. This trend was consistent between mice and RA patients.

In summary both NK cells and ILCs are major contributing cells to RA pathogenesis. Deviations from normal NK and ILCs functions and composition may influence the severity of RA manifestation.

## Innate immune signaling drives RA pathogenesis

### Toll-like receptors (TLRs)

TLRs play a central role in regulating innate immunity and in recent years their role in autoimmune diseases such as RA is becoming clearer. TLRs represent a family of pattern recognition receptors (PRRs) and are the front-line sensors of danger signals that are released following injury or infection [14]. It is postulated that harmful stimuli triggered by injury, infection, stress, hypoxia or cell death, ignite tissue damage and release of endogenous TLR ligands in the periphery. TLR2, TLR4, TLR5, TLR7 and TLR8 are highly expressed by RA peripheral blood monocytes and synovial macrophages, and ligation by their exogenous and endogenous ligands promotes inflammatory responses [65]. The functions of TLR2 and TLR4 have been extensively studied in RA through the use of in vitro systems and experimental models. Several Pathogen-Associated Molecular Patterns (PAMPs), such as lipoolarabinomannan (LAM), lipopolysaccharide (LPS), lipoteichoic acid (LTA), peptidoglycan (PGN), and other glycolipids,

glycoproteins and lipoproteins, are activators of TLR2, while TLR4 is activated by LPS, as well as fungal mannan and glucuronoxylmannan [65]. TLR2 expression is increased on peripheral blood CD16<sup>+</sup> monocytes as well as on synovial fluid (SF)-macrophages of RA patients compared with health controls [66]. Similarly, another group showed that TLR2 and TLR4 expression was higher on CD14<sup>+</sup> SF macrophages of RA patients compared with healthy controls, and that TLR2 and TLR4 ligation led to increased activation of SF macrophages in vitro [67]. A study investigating blood taken from RA patients showed significantly increased expression levels of TLR2 and IL-6 compared to healthy controls. However, TLR4 expression was not significantly different between RA and healthy controls [68] indicating a more critical role for TLR2 than TLR4 in RA pathogenesis. Indeed, the importance of TLR2 in RA pathogenesis was highlighted by its ability to induce synovial fibroblast migration, invasion and MMPs production in vitro [69]. Although TLR2 and TLR4 function has been implicated in RA pathogenesis, there is conflicting information regarding association of SNPs of TLR2 and TLR4 with severity of RA [70,71].

TLR5, which recognizes bacterial flagellin components, correlates with TNF levels and RA disease activity as measured by the DAS28, a clinical assessment of joint inflammation and other factors [72]. Endosomal TLR3 is activated by viral double stranded (ds) RNA and can be activated by dsRNA coming from necrotic cells in the RA synovium [73]. Altogether, the literature suggests that the pathogenic effect of TLR3 is due to its differential expression in RA compared to normal fibroblasts and its presence on myeloid cells is less significant for disease progression [74,75]. Both TLR7 and TLR8 recognize ssRNA [76]. While expression of TLR7 is enhanced by IL-17 and IL-8, LPS and IL-1 are responsible for increasing the TLR8 levels in RA monocytes and macrophages [76]. TLR9 recognizes internalized bacterial DNA and non-methylated CpG oligonucleotides [77]. Inhibition of TLR9 expression in a rat model of RA along with studies involving TLR9 deficient mice have shown delayed onset of arthritis and an overall reduced level of bone erosion [78].

Regulation of TLR signaling pathways is very important for disease outcomes [30,65]. TRAF1 in the TRAF1-C5 region is the third most strongly RA-associated locus in ACPA positive RA according to GWAS data [79]. A follow up study showed that monocytes from healthy donors with disease associated TRAF1 SNPs were over-responsive to TLR4 stimulation and produced more pro-inflammatory cytokines. Mechanistically, the study revealed an unexpected role for TRAF1 in its negative regulation TLR signaling by interfering with linear ubiquitination of IKK $\gamma$  (NEMO) [80].

TLR2 and TLR4 expression levels can be regulated by microRNAs (miR), thereby affecting disease outcome. In RA patients, fibroblast-like synoviocytes (FLS), which play a central role in osteoarticular destruction, express several TLRs but strongly upregulate TLR2 expression. Transfection of this key effector cell with miR-19a/b led to reduced TLR2 expression and thus an overall reduction in the production of IL-6 and matrix metalloproteinase 3 [81]. Similarly, Jian et al. showed that miR-26a directly targets TLR3 leading to reduced inflammation and bone erosion in animal models of RA [82].

In conclusion, binding of TLR ligands to responsive cells can lead to chronic inflammation that results in cartilage and bone destruction in RA. Novel approaches are currently being tested to target TLR expression and signaling as potential treatments for RA.

### Inflammasomes

IL-1 $\beta$  and IL-18 are two potent cytokines that are released as a part of the innate defense mechanism to invading pathogens. However, excessive release of these cytokines, particularly IL-1 $\beta$ , is associated with autoimmune disorders and septic shock [83]. Therefore, it's not surprising that its secretion is tightly regulated, where it requires a second independent signal to be secreted. As with other cytokines, the first signal entails transcription of the IL-1 $\beta$  gene, but the gene product, pro-IL1 $\beta$  is not active. The second signal entails activation of the inflammasome, a multimeric protein complex typically comprised of a danger sensing receptor (NLR), an ASC adaptor protein and the zymogen pro-caspase-1 [84]. Following inflammasome assembly, caspase-1 is activated and directs the cleavage of pro-IL1 $\beta$  and pro-IL18 into active and secreted IL-1 $\beta$  and IL-18, respectively. Due to the various complexities of danger signals, many different types of inflammasomes have evolved — each with its own danger receptor. The canonical inflammasome can be nucleated by the NLR members NLRP1, NLRP3 and NLRC4 or by the ALR member AIM2. Of the inflammasomes that have been described, the NLRP3 inflammasome stands out for the large number of “molecular patterns” it responds to and the large number of human diseases in which it has been implicated [85]. Malfunctioning of the NLRP3 inflammasome is a driver of autoimmune diseases like gout, RA and lupus [86]. Moreover, IL-1 $\beta$  has long been implicated in cartilage erosion and the prevention of chondrocyte matrix formation (reviewed in (Abramson and Amin, 2002)).

Dysregulated activity of the NLRP3 inflammasome has been associated with inflammatory joint diseases like osteoarthritis and gout as well (reviewed in Ref. [87,88]). However, the relationship between the NLRP3 inflammasome and RA is not conclusive. Various mouse models of RA have been employed to study the pathogenesis of RA and the efficacy of RA suppressive drugs. Zhang et al. employed a collagen induced arthritis (CIA) model and showed that increased synovial and serum NLRP3 expression increased in the early onset of CIA and directly correlated with disease severity [89]. This conclusion was recently confirmed by another group indicating increased inflammasome activity in the synovia of RA patients and CIA mice. Increased inflammasome activity led to increased inflammatory signature associated with RA, whereas inhibition of inflammasome activity by MCC950 led to significantly lower inflammation [90]. This is likely due to the local inflammatory nature of this model compared to CIA. Moreover, in the spontaneous RA model, A20<sup>Myel-KO</sup>, myeloid-cell specific deletion of the RA susceptibility gene A20/Tnfrsf3, led to increased NLRP3 mediated caspase-1 activation and IL-1 $\beta$  secretion, which positively correlated with increased arthritis pathology in these mice. This phenotype was rescued by NLRP3, caspase-1 or IL-1 receptor (IL-1R) deletion [91]. Choulaki et al. demonstrated that the NLRP3 inflammasome was overexpressed and overactivated in whole blood cell

samples of human RA patients when compared to healthy controls [92]. On the other hand, a previous study utilizing antigen induced arthritis (AIA) model showed that RA pathogenesis is dependent on ASC but independent of other inflammasome components such as NLRP3, NLRC4 and caspase-1 [93]. This discrepancy might be due to a context dependent role for NLRP3 inflammasome or because other inflammasome, like NLRP1, play a more important or consistent role in RA pathogenesis (reviewed in Ref. [88]).

In addition to innate immune cells, T<sub>H</sub>17 cells of RA patients exhibited enhanced NLRP3 activation, which correlated with increased IL-1 $\beta$  and IL-17 levels in RA sera. Inhibition of NLRP3, caspase-1 and IL-1R led to decreased T<sub>H</sub>17 differentiation [94].

These studies showcase the possibility of inflammasomes as a therapeutic target in RA patients. However, important mechanistic insights and key intracellular signaling pathways leading to inflammasome activation must be elucidated prior to this becoming a reality.

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### Innate immune system as a treatment target for RA

Over the past two decades, the treatment of RA has been revolutionized by advances in the understanding of its pathologic mechanisms and the development of drugs that target them. Biological agents that have been approved for RA therapy include antibodies that target TNF, IL-6 or IL-1 $\beta$  activity as well as therapeutics that block T cell function or deplete B cells. We have already discussed some of these potential therapies that target monocytes, macrophages and neutrophils above. However, more recent advances in the field of RA treatment enables novel therapeutic approaches that target TLRs, NLRP3 inflammasomes, ILCs and DCs. In spite of all this, there are subsets of RA patients that do not respond to the aforementioned strategies. RA is a complicated disease caused by many genetic and environmental factors. Therefore, the need persists for novel therapeutic approaches that are more tailored, effective and less expensive.

A number of strategies can be utilized to abrogate TLR driven inflammatory responses. These strategies include: 1) use of soluble decoy receptors or neutralizing antibodies that abolish the ligand and receptor binding, 2) suppressing the production of endogenous ligands or TLR expression levels, 3) inhibiting TLR linked downstream pathways and 4) inhibiting TLR expression, in part through mi-RNAs. As TLRs play an integral role in the innate immune response, ‘controlling’ the overall contribution of these receptors can have major impact on RA manifestation. Specific receptor targeting can lead to diminished inflammation in RA patients.

Given the complex roles that DCs play in RA pathogenesis, therapies targeting them are being developed to either block the immunogenic or enhance tolerogenic functions of DCs [95]. In a recent study involving a collagen-induced arthritis (CIA) mouse model, treatment of bone marrow-derived DCs (BMDCs) with the TNF blocker, etanercept, led to delayed onset of arthritis and reduced arthritis symptoms [96]. Following LPS stimulation, etanercept-treated BMDCs failed to mature and migrate to local lymph nodes resulting in overall

reduced inflammatory cytokine production [96]. On the other hand, therapies exploiting the tolerogenic abilities of DCs are currently under development. Autologous ‘Tolerogenic’ DCs (ToIDC) are derived from RA patient's own myeloid precursors *ex vivo*, loaded with antigen, and manipulated to suppress autoreactive T cells and then injected back into the patient's synovial joints. A Phase I clinical trial employing ToIDCs showed the feasibility of this approach [97]. Additional research is exploring how to optimize this process. For instance, maturation of ToIDC *in vitro* after stimulation with PAMPs (Monophosphoryl Lipid A (MPLA) or lipopolysaccharide (LPS)) leads to enhanced stability of the tolerant phenotype as well as the ability to suppress CD4 T cell responses in an experimental arthritis model [98]. Another group showed that preloading ToIDC with heat shock proteins (HSP40-, HSP60- and HSP70-derived peptides) may be a promising tool to restore immune tolerance in RA patients by converting HSP-specific CD4 T cells into anti-inflammatory T<sub>Reg</sub> [99].

### Future perspectives

Recent studies discussed here demonstrate that major advances have been made towards our understanding of how innate immune cells and signaling drive RA pathogenesis and showcase the complexity of this disease. Specific therapeutic targeting the innate immune system as well as its effectors and components can lead to reduced incidence and may improve quality of life for RA patients. This, however, requires additional mechanistic studies that investigate how metabolic and epigenetic reprogramming of innate immune cells shape their role in RA pathogenesis. This can potentially shed some light into how environmental factors like smoking, physical activity and infections alter the susceptibility to RA.

Given the heterogeneous nature of innate immune cells, especially monocytes, macrophages and dendritic cells, major advances in single cell RNA sequencing (scRNAseq) technology may be instrumental in identifying the contribution of the various innate immune cell subsets to RA pathogenesis. Such studies can potentially delineate the evolving role of these subsets throughout the course of the disease. Finally, studies examining how macrophage polarization in the inflamed joints as the disease progresses can offer significant insights into the complex and central role of these cells in RA.

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### Conflicts of interest

The authors have declared that no competing interests exist.

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