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Review Article

Receptor targeting drug delivery strategies and prospects in the treatment of rheumatoid arthritis

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Abstract

Rheumatoid arthritis (RA), a chronic inflammatory disease, is characterized by cartilage damage, bone tissue destruction, morphological changes in synovial fluids, and synovial joint inflammation. The inflamed synovial tissue has potential for passive and active targeting because of enhanced permeability and retention effect and the existence of RA synovial macrophages and fibroblasts that selectively express surface receptors such as folate receptor β, CD44 and integrin αVβ. Although there are numerous interventions in RA treatment, they are not safe and effective. Therefore, it is important to develop new drug or drug delivery systems that specifically targets inflamed/swollen joints but attenuates other possible damages to healthy tissues. Recently some receptors such as toll-like receptors (TLRs), the nucleotide-binding oligomerization domain-like receptors, and Fc-γ receptor have been identified in synovial tissue and immune cells that are involved in induction or suppression of arthritis. Analysis of the TLR pathway has moreover suggested new insights into the pathogenesis of RA. In the present paper, we have reviewed drug delivery strategies based on receptor targeting with novel ligand-anchored carriers exploiting CD44, folate and integrin αVβ as well as TLRs expressed on synovial monocytes and macrophages and antigen presenting cells, for possible active targeting in RA. TLRs could not only open a new horizon for developing new drugs but also their antagonists or humanized monoclonal antibodies that block TLRS specially TLR4 and TLR9 signaling could be used as targeting agents to antigen presenting cells and dendritic cells. As a conclusion, common conventional receptors and multifunctional ligands that arte involved in targeting receptors or developing nanocarriers with appropriate ligands for TLRs can provide profoundly targeting drug delivery systems for the effective treatment of RA.

Keywords: Delivery; Drug; Inflammation; Receptor; Rheumatoid arthritis; Target.

1. INTRODUCTION

Rheumatoid arthritis (RA), a chronic inflammatory disease with a prevalence of about 0.5 to 1% in the world, is associated with symptoms such as pain and stiffness in multiple joints, one-third of the patients, however, initially experience symptoms at just one location or at a few scattered sites (1,2). In the majority of patients, RA may result in a variety of extra articular manifestations, such as fatigue, subcutaneous nodules, lung involvement, pericarditis, peripheral neuropathy, vasculitis, and hematologic abnormalities. The disease course may range from self-limiting oligoarticular illness with minimal joint damage to a sustained polyarticular, synovial inflammation resulting in progressive cartilage destruction and bone erosion (2,3). In RA, inflammation of the synovial tissue is the main character at the cellular level; the synovium becomes infiltrated with T cells, dendritic cells (DC), macrophages, fibroblasts, mast cells, neutrophils and B cells. Morphological changes in synovial fluids including formation of tubule-like structures, induction of angiogenesis, changes in synovial blood vessel density, and alterations in endothelial proliferative responses take place.

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Additionally, the vascular endothelial growth factor (VEGF) levels and the key pro-angiogenic factors are increased in RA with disease duration of less than 2 years, and predict subsequent joint destruction suggest that angiogenesis may be an early event in RA progression (4-6). The volume of synovial fluid, nurturing and lubricating the joints, increases which results in joint swelling and pain.

The pathogenic mechanisms of synovial inflammation include a complex interaction of genetic, environmental, and immunologic factors that produces deregulation of the immune system and a breakdown in self-tolerance. In the development of RA, genetic predispositions together with environmental factors are possible triggers, with following synovial T cell activation. Antigen presenting cells (APCs) *via* interactions with T cell receptor and class II major histocompatibility complex-peptide antigen with co-stimulation through the CD28-CD80/86 pathway as well as other pathways activate CD4+ T cells. In theory, after binding of ligands to toll-like receptors (TLRs), the APCs inside the joint may stimulate synovial CD4+ T cells to differentiate into T-helper (Th) 1 and Th17 cells, each with their distinctive cytokine profile. CD4+ Th cells in turn activate B cells, some of which are intended to differentiate into autoantibody-producing plasma cells. The immune complexes of rheumatoid factors and anti-cyclic citrullinated peptides antibodies may be formed inside the joint, activating the complement pathway and amplifying inflammation. The pro-inflammatory mediators such as tumor necrosis factor alpha (TNF-α) are secreted by synovial macrophages and fibroblasts which stimulates T effector cells. TNF-α stimulates the production of other inflammatory mediators, such as interleukin 1 (IL)-1, IL-6, and granulocyte-macrophage colony-stimulating factor, and has a critically important function in regulating the balance between bone destruction and formation. TNF-α stimulates osteoclastogenesis by up regulating the expression of dickkopf-1, which can inhibit the Wnt pathway. Wnt is a soluble mediator that promotes osteoblastogenesis and bone formation (2,7).

Non-steroidal anti-inflammatory drugs (NSAIDs) typically support the treatment strategies for RA and corticosteroids controlling pain and inflammation; the disease-modifying anti-rheumatic drugs (DMARDs) which prevent joint damage. Biologic response modifiers (biologicals) using for selective inhibition of specific molecules involve five different modes of action, including TNF inhibition (infliximab, etanercept, adalimumab, certolizumab, golimumab), T cell co-stimulation blockade (abatacept), IL-6 receptor inhibition (tocilizumab, tofacitinib, baricitinib), B cell depletion (rituximab), and IL-1 inhibition (anakinra) of the immune system (8,9).

Unfortunately, extra articular adverse effects and toxicity with these drugs particularly in long-term use and in large doses and due to the unselective distribution of these drugs and lack of specificity towards rheumatic organs/tissues are a major concern in clinical use of these agents. Moreover, short half-lives and inadequate drug concentrations in the inflamed joints and areas requires large and frequent dosing resulting in severe side effects and high cost (10). Direct intra-articular injection to the infected joints might be a possible solution to avoid the off-target toxicity of these drugs; however, this approach still has many limitations, such as frequent joint needling, risk of infection, joint disability, and intolerance of the patients. Above all, development of novel and effective treatments *via* joint-targeting drug delivery may be an attractive option. This could be achieved with strategies combining nanotechnologies passive targeting and ligand-receptor mediated active targeting. Colloidal targeted drug delivery systems anchored with appropriate ligands for specific receptor overexpressed on cells involved in pathogenesis of RA target drugs specifically to the site of inflammation. This could take place through a process of extravasation through leaky vasculature and subsequent inflammatory cell-mediated sequestration (ELVIS), which is similar to the classic enhanced permeability and retention effect as observed in tumor tissues (10) and efficient

uptake of the particles into the diseased cells *via* a ligand-receptor mediated endocytosis. The two primary cell types existing in the pannus tissue, RA synovial macrophages (RASMs) and RA synovial fibroblasts (RASFs) selectively express surface receptors such as folate receptor (FR)-β, CD44 and integrin αVβ that are candidates for conventional active targeting (11-14). In addition to these, another viable target for drug delivery in RA is E-selectin adhesion molecule (15). Thus, in the current paper, we reviewed, in brief, the potential targets (FR-β, CD44, and integrin αVβ), the TLR family integral membrane bound receptors, the nucleotide-binding oligomerization domain-like receptors (NOD-like receptors), and Fc-γ receptor which could be utilized to facilitate the effective specific delivery of drugs to the inflamed synovium. Different drug delivery systems so far been used for targeting these receptors in RA including the liposomes, nanoparticles, and polymeric micelles are also discussed.

2. CONVENTIONAL ACTIVE TARGETING APPROACHES IN RA TREATMENT

2.1. FR-β-mediated active targeted drug delivery system

Among three major forms of FR binding to folic acid with high affinity, FR-β has been identified as a viable candidate for active targeting to RASMs. Following binding, rapid endocytosis occurs and a fraction of the receptors is delivered into a low pH compartment where dissociation of the vitamin from its receptor is promoted (16-18).

A study showed that activated (but not resting) synovial macrophages from patients with RA have a functionally active FR-β. This study indicated that compared to monocytes or macrophages from healthy individuals, both FR-β mRNA and the expressed protein are not only more abundant in activated monocytes and RASMs, but the FR-β on the activated cell surfaces can bind folate-linked fluorophores with high affinity (19). Thus, activated macrophages and RASMs can be selectively targeted with folate conjugates in arthritic joints, and has a potential to open up new possibilities for the diagnosis and treatment of RA (20). For more information, studies that have used folate as targeting ligand for FR-β are listed in Table 1.

2.2. CD44 receptor-mediated active targeting drug delivery systems

RASFs have an additional active targeting receptor on the cell surface adhesion molecule CD44 namely the hyaluronic acid receptor (31,32). Several studies have indicated that CD44 is a critical pathogen molecule in RA that is upregulated in the pannus tissues relative to healthy tissues, facilitating inflammatory cell migration and signaling activation of lymphocytes (33). `Some studies that have used hyaluronic acid as a targeting agent for CD44 receptor are summarized in Table 2.

2.3. Vitronectin receptor-mediateda active targeting drug delivery systems

The integrin αVβ3, also known as the vitronectin receptor, consists of a 125 kDa αV subunit and a 105 kDa β3 subunit that is overexpressed by both angiogenic endothelial cells and RASMs (45,46). Integrin αVβ3 has distinct functional properties that are mediated through interactions with a variety of extracellular matrix (ECM) proteins including osteopontin, fibronectin, fibrinogen, thrombospondin, proteolysed collagen, and von Willebrand factor. The interactions with ECM have functional effects on the expression of other cell-surface receptors such as VEGFR1, VEGFR2, fibroblast growth factor receptors 1 and 2 on the endothelial cell surface (47). VEGFR1 and VEGFR2 are two types of tyrosine kinase receptors associated with angiogenic activity of VEGF, a potent inducer of synovial proliferation that is synthesized by various cell types such as macrophages, fibroblasts, vascular smooth muscle cells and synovial lining cell in the synovium (48,49). A recent study showed that treatment with anti-VEGF-RII had no effect in RA, but anti-VEGF-RI reduced the intensity of clinical manifestations and, based on qualitative and semi quantitative histological analyses, prevented joint damages (50).

Table 1. Folate drug delivery strategies for rheumatoid arthritis.

Carrier systems	Materials	Drug agents	References
Nanoparticles	PAMAM dendrimer folate	Indomethacin	(21,22)
	PAMAM dendrimers folate	Methotrexate	(23)
	Chitosan folate	DNA	(24)
	Lipid PEG-folate	NF-κB Decoy	(25)
	Calcium phosphate/liposome-based hybrid nanocarrier-folate	NF-κB-targeted siRNA and methotrexate	(26)
	PK3 (pH-sensitive polymer) folate-PEG-	siRNA	(27)
	PK3, folate-PEG-PLGA, egg PC, and Sta-	Methotrexate	(28)
Micelle	Natural chlosterol-polysialic acid-folate	Dexamethasone	(29)
Liposome	Folate conjugated double liposomes	Prednisolone and methotrexate	(30)

PAMAM, Polyamidoamine; PEG, polyethylene glycol; NFκB, nuclear factor kappa B; PLGA, poly(lactic-co-glycolic acid); PC, phosphatidylcholine.

Table 2. Hyaluronan drug delivery strategies for rheumatoid arthritis.

Carrier systems	Materials	Drug agents	References
Nanoparticles	HA nanoparticles	γ-Secretase inhibitor (DAPT)	(34)
	PEI, egg PC, PCADK, HA	Dexamethasone	(35)
	SLNs coated with HA	Prednisolone	(36)
	HA-gold nanoparticle	Tocilizumab	(37)
	HA-MTX conjugate	Methotrexate	(38)
	PEG-TRAIL in 1% HA	TRAIL	(39)
	MTX, peptide, linker, HA	Methotrexate	(40)
	HA-CA conjugate with an acid-labile ketal linker	Methotrexate	(41)
	Click-crosslinked HA	Methotrexate	(42)
	HA and Pluronic F-127	Piroxicam	(43)
Liposome	HA-conjugated pH-sensitive liposomes	Prednisolone	(44)

HA, Hyaluronic acid ; PEI, polyethylenimine; PC, phosphatidylcholine; PCADK, poly (cyclohexane-1,4-diyl acetone dimethylene ketal);.CA, 5-cholanic acid; SLN, solid lipid nanoparticle; MTX, methotrexate; PEG, polyethylene glycol; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

Another study showed that addition of anti-αVβ3 and αVβ5 antibodies such as vitaxin to the culture medium blocked the increased expression of VEGFR1 and other receptors (47). In addition, the role of osteopontin, an ECM protein containing Arg-Gly-Asp (RGD) sequence that interacts with αVβ3 integrins, has been examined in an experimental RA model.

The results showed that osteopontin deficiency prevented surface destruction, loss of proteoglycan in the articular joint cartilage, and swelling of the joints. These preclinical studies illustrate that αVβ3 is an appropriate target for the treatment of RA and related diseases (51). Table 3 shows some studies where RGD has been used as a targeting agent for vitronectin receptors.

2.4. Active drug delivery systems targeting E-selectin receptors

E-selectin, a member of selectin family expressed in both acute and chronic inflammation, has been considered as a suitable candidate for drug targeting. Some studies have shown that cytokines IL-1, TNFα, and bacterial lipopolysaccharide (LPS) could stimulate the expression of E-selectin having a high expression in neovasculature and prostate cancer (56-58). The fucosylated glycoproteins (ESL1, for E-selectin ligand) or sialomucin glycoproteins (PSGL-1 for P-selectin glycoprotein-1) that have sialylated carbohydrate moieties (sialyl-Lewis x, sLex) epitopes are natural ligands of E- and P-selectin, and these selectin ligands are known to be present on circulating leukocytes plasmatic membrane (59,60).

Table 3. RGD drug delivery strategies for rheumatoid arthritis.

Carrier systems	Materials	Drug agents	References
Nanoparticles	RGD-PLGA	STAT1 siRNA	(52)
	RGD-attached Au half-shell nanoparticles	Methotrexate	(53)
Liposome	RGD-PEG-liposomes	Dexamethasone phosphate	(54)
Micelle	RGD-PEG-PLA	Methotrexate and nimesulide	(55)

RGD, Arginyl-glycyl-aspartic acid; PLGA, poly (lactic-co-glycolic acid); PEG, polyethylene glycol; PLA, polylactic acid.

Recently, synthetic analogues of the natural ligands have been investigated that could attenuate inflammation and some of these analogues showed higher affinity to the targets than natural ligand, making them very attractive molecules for active drug targeting (59,61,62). Dexamethasone (DEX)-anti-E-selectin immunoconjugate and anti-E-selectin immunoliposomes containing DEX were prepared and compared. The results showed that though DEX-anti-E-selectin was internalized to a larger extent than the anti-E-selectin immunoliposomes, the high drug-loading capacity of the liposomes would allow a larger quantity of DEX for intracellular delivery. Thus, both conjugate and liposomes accumulated in the activated endothelial cells of inflamed murine skin (63,64). Another study developed surface-modified liposomes with the polysaccharide Sialyl Lewis X, which is known to bind selectively to E-selectin. The results showed these particles accumulated within inflamed regions when administered intravenously to arthritic mice (65).

3. POTENTIAL NEW ACTIVE TARGETING APPROACHES IN RA TREATMENT

Nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) are pattern-recognition receptors (PRR) similar to TLRs. While TLRs are transmembrane receptors, NLRs are cytoplasmic receptors that play a crucial role in the innate immune response by recognizing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). Upon PAMP or DAMP detection, NLRs trigger a signaling cascade that activates innate antimicrobial responses *via* the production of pro-inflammatory cytokines, chemokines, type I interferons (IFNs) and antimicrobial peptides, and finally these chemical signals activate B and T cells, thus linking the innate and adaptive immune responses. TLRs and NLRs are the well characterized PRRs and subjects of considerable investigation (66,67). In addition to innate immune recognition mechanism, most of the studies reported immune complexes (IC) found in the circulation and afflicted tissues and organs engaged with immune cells and affected Fc receptors. Finally, they can trigger cell recruitment and activation, localized inflammation, adaptive immunity and tissue pathology (68,69).

3.1. Toll-like receptors

TLRs have several key roles in pathological process of RA. First, they identify viral and bacterial pathogens such as herpes virus and mycoplasma, which have long been suspected to be associated with RA, as well as several endogenous proteins found in abundance in the RA joint such as heat shock proteins, fibronectin fragments, necrotic cells, hyaluronan oligosaccharides and IgG-chromatin complexes (70,71). Second, TLRs are able to initiate or promote strong immune and inflammatory responses that mentioned above. Finally, these receptors are able to potently activate NF-κB and induce the production of various cytokines and chemokines including TNF and IL-1, two validated targets for the treatment of RA (71).

The TLRs family are integral membrane bound receptors that are vital for innate immunity and help to shape the adaptive immune response in humans which composed of 10 members, TLR1 to TLR10 (72). Based on their localization, TLRs are dichotomized into groups including TLR1/2/4/5/6/10

that are on the cell membrane and TLR3/7/8/9 that are on the membranes of intracellular compartments such as endosomes and endolysosomes. Immune cells, mast cells, and epithelial cells that are exposed to the external environment such as mouth, lungs, and gut (73) predominantly express these receptors. In patients with RA, important changes in synovium include expansion of the synovial intimal lining composed of fibroblast-like synoviocytes (FLS) that overexpress TLR2/3/4/7and produce a high amount of IL-6 and metalloproteinase 3, and macrophage-like synoviocytes that produce a large panel of pro-inflammatory cytokines in response to TLR overexpression. In addition to these, an abnormal presence of bacterial DNA and bacterial peptidoglycans has been reported in joints of patients with RA (74,75), and active TLR4 ligands are increased in the serum and synovial fluid of RA patients (76).

3.1.1. The role of TLR2 in RA

Recent studies showed that in patients with active RA, the D16 monocytes that express higher levels of TLR2 are expanded in association with a cytokine spillover from the inflamed joints. These results indicated macrophage colony-stimulating factor and IL-10 induced CD16 monocytes and synovial tissue macrophages with high TLR2 expression, and their production of TNF could be stimulated by endogenous TLR ligands such as Hsp60 and FcRIIIA ligation by small IC in RA joints (77).

3.1.1.1. TLR2 agonist and antagonist

SMP-105 is a TLR2 agonist that consists of cell-wall skeleton components, such as mycolic acids, and peptidoglycans from *Mycobacterium bovis* (strain BCG/ Tokyo), approved for the treatment of bladder cancer and it has shown strong adjuvant and antitumor activities. In mice, this agonist induced NF-κB, TNF-α and IL-6 production in a TLR2-dependent manner. These results suggest that the activation of TLR2 by SMP-105 sufficiently enhanced immune responses such as the number of IFN-γ -producing cells and cytotoxic T lymphocytes, and prevented the growth of the tumors (78).

A recent study used whole tissue synovial explant cultures *ex vivo* (which closely reflect the *in vivo* environment) and RA mononuclear cells to demonstrate that Pam3CSK4, a TLR1/2 agonist, significantly increased release of key cytokines. The result indicated Pam3CSK4 significantly upregulated IL-6 and -8 in RA peripheral blood mononuclear cells (PBMC), RA synovial fluid mononuclear cells (SFMCs) and RA synovial explant cultures ($P < 0.05$). In this study, to assess an anti-TLR2 antibody, the culture of RA PBMC and SFMC were incubated with Pam3CSK4 (TLR1/2 ligand) and anti-TLR2 antibody (OPN301) or an immunoglobulin G (IgG) (1 μg/mL) matched control. The result showed OPN301 significantly inhibited Pam3CSK4 induced IFNg, IL-1b, IL-6, TNF-α, and IL-8 cytokine production in RA SFMCs and PBMC (all $P < 0.05$) compared to IgG isotype control. The result of RA synovial explants demonstrated that OPN301 penetrated in the synovial tissue, localizing to the lining layer and perivascular region and significantly suppresses spontaneous release of pro-inflammatory cytokines compared to Adalimumab, a well-established TNF blocking therapy. These results indicated that TLR2 promotes pro-inflammatory and destructive processes in RA and further support the rationale of using a TLR2 therapeutic blockade (79). OPN-305 is another TLR2-specific monoclonal antibody inhibiting TLR2-mediated pro-inflammatory cytokine production and is being tested for the potential treatment of inflammatory diseases such as systemic lupus erythematosus and RA (80,81).

3.1.2. The role of TLR3 in RA

TLR3 is an endosomal TLR expressed in DC and recognizes dsRNA (82,83). A study indicated RNA that is released from RA necrotic synovial fluid cells can activate TLR3 on RASFs (84). Although nucleic acids are readily degraded outside of the cell, in the RA joint, other molecules may protect them. In RA synovial fluid, there is human cationic antimicrobial peptide, LL-37, at high level that binds RNA and protects it from degradation (85,86). In addition to

this, mimicking the hypoxic conditions of the *in situ* joint has shown to exacerbate cytokine and matrix metalloprotease production following activation of TLR3 in RASFs (87). To determine the role of TLR3 signaling in angiogenesis in the rheumatoid synovium, FLS were isolated from RA synovial tissues and stimulated with the TLR3 ligand, poly-I:C. The result showed the expression levels of two pro-angiogenic molecules, VEGF and IL-8, were upregulated by TLR3 ligation in human RA FLS. These results suggest that the activation of TLR3 in RA FLS promotes the production of proangiogenic factors; so targeting the TLR3 pathway may be a promising approach to prevent pathologic angiogenesis in RA (88).

In a rat model of pristane-induced arthritis (PIA) TLR3 was significantly upregulated in splenocytes following pristane injection and stimulation of TLR3 with poly-I:C exacerbated disease. In the same study has also shown that downregulation of TLR3 with siRNA ameliorated the disease, pointing to a key role in disease progression (89). In another similar study, TLR3 was upregulated during methotrexate administration in splenocytes from both collagen-induced arthritis (CIA) and PIA rat models. The results indicated methotrexate could inhibit both disease symptoms and the increase in TLR3 expression. Therefore, overexpression of splenic TLR3 is strongly associated with arthritis in rats, which suggests that TLR3 should be a most vital TLR in spleen to regulate the initiation and development of experimental arthritis and may be an interesting therapeutic opportunity for human RA (90).

3.1.3. The role of TLR4 in RA

TLR4 is a cell membrane receptor that increases sepsis, RA, ischemia/ reperfusion injury, and allergy (91). Several endogenous ligands such as sHSPαA crystallin, HSPB8 or Tenascin C that are present in the synovial membrane activate the innate immune system bind to TLR4 and may promote joint inflammation confirming a significant role for TLR4 in the pathogenesis of RA (92-94). Pierer *et al*. demonstrated that TLR4 deficient DBA mice develop collagen-induced arthritis with lower incidence and decreased severity. In this study, TLR4-deficient mice were virtually protected from cartilage destruction, and infiltration of inflammatory cells was reduced compared to wild type mice. Parallel with the reduced clinical severity, lower IL-17 and lower anti-anticyclic citrullinated peptides antibody were found in the TLR4 deficient mice. This study supports the role of TLR4 in the dissemination of joint inflammation and destruction, and it indicated a link between TLR4 stimulation and the adaptive autoimmune response (94).

3.1.3.1. Ligands potentially used in TLR4 targeting

3.1.3.1.1. Hyaluronan

Some studies have indicated hyaluronan in addition to coupling with CD44 receptor, could have various effects on the inflammatory response related to its molecular size. Low molecular weight hyaluronic acid upregulated the expression of TLR4, induced MyD88 and the inflammation mediators in untreated chondrocytes, and enhanced LPS effect in LPS-treated cells. However, the medium and high molecular weight hyaluronic acid exerted no activity in untreated cells and only the high molecular weight hyaluronic acid reduced the LPS effects. These findings suggest that the regulatory effect of hyaluronic acid at each molecular weight on NF-kB activation may be depend on the interaction between hyaluronic acid and TLR4 and hyaluronic acid may thereby regulate pro-inflammatory activity *via* its different state of polymerization (95-98). Another study reported that inhibition of endogenous hyaluronic acid degradation during arthritis might lead to reducing TLR4 and CD44 activation and the inflammatory mediators' response (96). Furthermore, polymeric structure of hyaluronic acid seems able to mask active sites of TLR4, thereby preventing the binding of these receptors with PAMPs (99).

3.1.3.1.2. Monoclonal antibody

In a recent study, the first humanized monoclonal antibody, NI-0101, which blocks

TLR4 signaling independent of ligand type (i.e., exogenous/endogenous) and concentrations is administrated to healthy volunteers. NI-0101 administration inhibited *ex vivo* and *in vivo* LPS-induced cytokine release, prevented CRP increase and the occurrence of flu-like symptoms following LPS administration to healthy volunteers. Interaction of NI-0101 with TLR4 and Fcγ receptor efficiently blocked TLR4 activation by citrullinated protein IC that present in synovial fluid of RA patient. These results strongly support the potential of TLR4 as a valid therapeutic target in RA, and provide an opportunity to evaluate specific endogenous TLR4 ligands as biomarkers of treatment response in phase 2 trial (100).

3.1.3.1.3. TLR4-antagonistic peptides

To develop TLR4 antagonists with rare off-target effects, recently small peptide inhibitors of TLR4/MD2-LPS and TLR4-antagonistic peptides (TAP) have been designed. In this study, TAPs-attributed TLR4-antagonism were initially evaluated through NFκB inhibition in HEK-blue hTLR4 and RAW264.7 cells, and then augmented by the downregulation of mitogen-activated protein kinases, NF-κB, IL-6, and suppression of the oxidative-stress products and inducible nitric oxide synthase (iNOS) in macrophages and human PBMC. Among these, TAP2 specifically suppressed the TLR4 and reduced LPS-elicited systemic cytokine. *In vivo* results suggested the small peptides specifically TAP2 have the potential to target TLR4 and to treat TLR4-mediated autoimmune diseases such as RA (101).

3.1.3.1.4. Glycol-split heparin nanoparticles

It has recently shown that unlike native heparin, heparins modified with lipids or incorporated into nanostructures act as selective TLR4 antagonists and has much greater anti-inflammatory activity. In a recent study, glycol-split non-anticoagulant heparin/D-erythro-sphingosine nanoparticles (NAHNPs) were synthetized and their therapeutic potential was examined in CIA animal model. The results showed that NAHNP significantly inhibited the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β in LPS-induced primary mouse macrophages and DC2.4 dendritic cell line. Treatment with NAHNP had a potent suppressive effect in the arthritis score and footpad swelling and levels of IgG1 and IgG2a antibodies against bovine type II collagen as well as levels of TNFα, IL-6, and IL-1β in knee joints and sera compared to control mice. Moreover, downregulation of the NF-κB signaling pathway was observed in NAHNP treatment, and histological examination revealed significant suppression of inflammatory cell infiltration, joint destruction and synovial proliferation in synovium compared with control mice (102). These results suggest that selective inhibition of TLR4-NF-κB signaling with NAHNP provides an effective therapeutic and targeting approach to inhibit chronic inflammation in RA.

3.1.3.1.5. Gum xanthan stabilized silver nanoparticles

Rao *et al*. reported oral administration of xanthan gum-stabilized silver nanoparticle (AgNP) with bergenin could target reactive oxygen species, cytokines, and TLR expression in adjuvant induced arthritis (AIA) model. They exhibited potent anti-arthritic activity with minimal arthritic scores, mild to moderate paw tissue swelling, reduced degenerative alterations along with mild articular changes and less influx of inflammatory cells in macroscopic X-ray and histological examination. Moreover, administration of bergenin and its NPs suppressed the levels of reactive oxygen species through their inhibitory effect against TLR (TLR2/4) and cytokine (IL-1b, IL-6, and TNFα) production. These results demonstrated xanthan gum-stabilized AgNP is a stable and promising multi-targeted therapeutic nano-cargo for bergenin and other drug delivery systems with efficient treatment of RA (103).

3.1.3.1.6. Opuntiol-coated silver and gold nanoparticles

Roome *et al*. reported that opuntioside-I (OPG) and opuntiol (OP), the chemical constituents isolated from *Oxalis dillenii*,

and their gold and silver NPs reduced AIA through the targeting TLRs (TLR2/4) and cytokines (IL-1β and TNF-α) expressions to validate their anti-inflammatory and immuno-modulatory response. Treatment with OP and OPG (10, 50, and 100 mg/kg) as well as OP-silver NPs and OP-gold NPs (0.5, 1, and 3 mg/kg) in arthritic rat showed minimal arthritic score and tissue swelling in ankle joints. In addition, the treatment groups significantly suppressed protein and mRNA expressions of TLRs (TLR2/4) and cytokines (IL-1β and TNF-α) levels comparing to arthritic control. These results suggested the tested compounds and nano-formulations successfully restored the disease progression in AIA rat owing to their anti-inflammatory potentials and can be considered for RA targeted therapy to address the utmost challenges of the disease (104).

3.1.4. The role of TLR5 in RA

TLR5 is a cell surface TLRs whose role in RA had not been fully known before 2012 (80). Comparison of *in vitro* differentiated macrophages from RA peripheral blood with normal peripheral blood showed elevated expression of TLR5 in RA synovial fluid macrophages and RA peripheral blood monocytes. Moreover, in RA synovial fluid monocytes, TLR5 is an important modulator of TNF-α and the expression of it on these cells strongly correlates with RA disease activity, and TLR5 expression has a feedback regulation with TNF-α. In addition, the TLR5 expression is regulated by IL-17 and IL-8 in RA macrophages and fibroblasts (80,105). In addition, the more responsibility of RA monocytes and macrophages to TLR5 ligation leads to pro-inflammatory response. These evidences suggest that expression of TLR5 may be a predictor for RA disease progression and targeting TLR5 may suppress RA (80,105).

3.1.5. The role of TLR7/8/9 in RA

TLR7/8 are endosomal TLRs are significantly expressed in RA monocytes at high level compared to macrophages. Ligation of a TLR7/8 agonist induces TNF-α production from macrophages, a key mediator of the disease process in RA. In support of pathological role of TLR7, a study on TLR7 (-/-) mice showed a reduction in clinical score, paw swelling, and number of paws affected when compared to wild type mice (106). The mice showed a significant decrease in IL-17/TH17 and an increase in Treg cells that are implicated in the control of inflammatory arthritis in animal models, pointing to a potential role of TLR7 in regulating T-cell plasticity or the balance between TH17 cell/Treg cell responses or both (106,107). Other studies indicated that activation of TLR7 with a low-dose of small synthetic ligand could induce tolerance of TLR2/7/ 9 signaling and suppress disease in a serum transfer model of arthritis (106,108). Another study showed that TLR8(-/-) mice developed spontaneous autoimmunity characterized by the increased serum levels of IgG2a, IgM, and autoantibodies against small nuclear ribonucleoproteins and dsDNA. The increased TLR7 levels being hyperresponsive to TLR7 activation was observed in DC obtained from these animals. In addition, concomitant TLR7/8 knockout, or individual TLR7 knockout mice did not show an autoimmune phenotype suggesting the potential for TLR8 to regulate TLR7 signaling in mice. Taken together these studies showed that TLR7/8 receptors may provide amenable targets for the development of new therapeutics in RA (106,109).

TLR9 is another endosomal TLRs that recognizes CpG-rich DNA or DNA conformation (110). In RA, TLR9 has a predominant role on B cells, where activation by DNA-containing IC has been shown to stimulate rheumatoid factor-positive auto reactive B cells (111). The data obtained from a recent study revealed involvement of TLR9 in the T cell-dependent phase of inflammatory arthritis. In this study, inhibition of TLR9 before disease onset significantly reduced arthritis and almost completely abolished bone erosion; and serum levels of IL-6, a-1-acid-glycoprotein, and rheumatoid factor were reduced in rats with PIA. Furthermore, in TLR9(-/-) mice, streptococcal cell wall-induced arthritis was reduced in the T-cell dependent phase, whereas, T cell-independent serum-transfer arthritis was

not affected. In precursor cells, the expression of TLR9 was higher than that of in mature osteoclasts and partial inhibition of osteoclastogenesis was achieved only by the TLR9 antagonist. These results demonstrate a key role of TLR9 in the T cell-dependent phases of inflammatory arthritis and suggest some role during osteoclastogenesis (112). Therapeutic application of TLR9 antagonists considered in the very early pre-clinical phases in persons at high risk for developing RA to prevent generation of autoimmune reactions leading to the inflammatory and deleterious processes in the joint characteristics of RA.

3.1.5.1. TLR7/8 /9 antagonists in RA treatment

Among conventional drugs used to treat RA, hydroxychloroquine is an antimalarial drug that is known to act as a TLR9 antagonist and to a lesser extent TLR7/8. A recent study has shown this drug also inhibited CpG-induced production of IL-6 and TNF-α in B cell subsets, and markedly suppressed the TLR9-mediated human B cell functions during inflammatory processes (113). Furthermore, selective serotonin reuptake inhibitors such as fluoxetine and citalopram, in addition to their antidepressant effects, have been reported to have anti-inflammatory effects. These drugs selectively inhibit endosomal TLR3/7/8/9 signaling, ameliorate disease in CIA, and suppress inflammatory cytokine production in human RA tissue (114).

3.1.5.1.1. TLR7/8/9 antibody-based antagonists

CPG-52364, an antagonist of TLR7/8/9, was initially used for the treatment of systemic lupus erythematosus (SLE), although it could also be useful in patients with RA (82). IMO-3100 (inhibiting TLR7/9) and IMO-8400 (inhibiting TLR7/8/9) are antagonists that inhibit signaling pathways and production of a broad range of cytokines, including TNF-α, IL-12, IL-6, IFN-α, IL-1β, and IFN-γ -induced protein -10, mediated by TLR7/8/9 in HEK293 mouse and human cell-based assays. In mice, these antagonists inhibited TLR7/9-mediated cytokine induction in a dose- and time-dependent fashion. In preclinical models of autoimmune diseases including CIA and lupus, these TLR antagonists inhibited Th1, Th17, and inflammation pathways and suppressed production of cytokines such as TNF, IL-12, IL-6, and IL-17 (115).

3.1.5.1.2. Attenuation of TLR9 signaling by gold nanoparticles

Although gold NPs have become one of the ideal nanomaterials for medical applications, the immunological effects of gold NPs are still of concern and require detailed investigation. Tsai *et al*. explored the immunological significance of gold NPs on TLR-mediated innate immunity in murine macrophages and raw 264.7. They found that gold NPs accumulated in lysosome after phagocytosis and they attenuate TLR9 signaling related to its molecular size. Among different sizes of gold NP, the smallest size (4 nm) is the most potent in the inhibition of the TLR9 signal because of the highest surface area to bind molecules in the lysosomal compartments such as the common DNA sensor. In relation to cytokine inhibition, gold NPs, especially in size of 4 nm, reduced TLR9 signaling and suppressed phosphorylation of NF-kB and Janus kinase as well as NF-kB activation. Thus, application of gold NPs as nanocarrier system may be useful in RA, because of TLR9 targeting potential and anti-inflammation effects (116).

3.1.5.1.3. Cationic dendronized polymer

The key auto-antigen in RA is cell free deoxyribonucleic acid (cfDNA) that is released from dead or damaged cells and is recognized by TLR, leading to activation of the innate immune system and chronic inflammation. Recently, Peng *et al*. developed a cationic molecular scavenger through a screening of cationic dendronized polymers, which could eliminate the cfDNA and inhibit the TLR recognition especially TLR9 and nucleic acid induced inflammation. This study demonstrated that toxicity, nucleic acid binding capacity and bio-distribution could be balanced to achieve maximum therapeutic effect with excellent control of molecular structure. Moreover, the suitable cationic polymer PCL-g-PAMAM Denpols effectively suppressed the joint swelling, synovial hyperplasia, and bone

destruction in CIA rat model. These results supported that the synthetic polymers offer new approaches for treatment and targeting drug delivery in RA (117).

3.2. NOD-like receptors

The NLR is one of the cytoplasmic PRRs involved in innate immune defense. The NLR family consists of 22 cytoplasmic proteins including the NOD and NALP subfamilies expressed in monocytes, DC, and non-hematopoietic cell types (67). Triggering of endogenous NOD leads to only moderate secretion of cytokines such as TNFα and IL-1 through activation of NFκB and mitogen activated protein kinase. NALP subfamilies contain three human inflammasomes, the NALP1 inflammasome activates caspase-5 and caspase-1; the NALP3 and IPAF inflammasomes, activate caspase-1 that is required for the processing and maturation of proinflammatory cytokines such as IL-1 and IL-18 leading to fever, joint pain and systemic inflammation in rheumatic diseases (118). Recently, the study of Guo *et al*. confirmed the role of NLRP3 inflammasome in RA by reporting an increased protein expression of NLRP3 and active caspase-1 in RA compared to osteoarthritis synovial tissue. According to this finding, they reported that using a small molecule inhibitor of the NLRP3 inflammasome, MCC950, ameliorated the synovial inflammation and cartilage erosion in a CIA mouse model. The level of IL-1β was also decreased in both serum and synovial tissue, while the levels of TNF-α and IL-6 remained unchanged. These results suggest that NLRP3-induced IL-1β release contributes to shape and stability of the immune/inflammatory processes in RA. Thus, inflammasome could be represented as a suitable pharmacological target for the management of RA (119). Yang *et al*. reported that oral administration of quercetin reduced arthritic scores and paw edema, decreased the joint levels of TNF, IL-6, PGE2, COX-2, iNOS, and Th17 cells, and increased the number of Treg cells in mice with CIA, through the inhibition of NLRP3 inflammasome activation (120).

3.3. Fc-γ receptors (FcγRs)

In humans, FcγRs are receptors for the Fc region of IgG possessing the activating receptors FcγRI, FcγRIIA, FcγRIIIA and FcγRIIIB and the inhibitory receptor FcγRIIB. FcγRI and FcγRIII activate cells *via* a common γ chain inducing severity of RA, but FcγRII suppresses RA through downregulation of antibody production and IC–triggered activation. The antibody affinities of most of these receptors are different due to their different molecular structures; however, FcγRI has higher affinity among FcγR in humans, which might be a desirable target for treatment of RA (8, 121). The FcγRIIA is an attractive target for the treatment of RA because of its expression on multiple immune cells that commence pathological inflammatory responses. Chen *et al*. showed that VIB9600 antibody could suppress IC-mediated cellular activation by inhibiting FcγRIIA, which provide alternative therapy for the treatment of autoimmune conditions. They also observed that VIB9600 could inhibit IC-induced TNFα and IL-6 production in RA (121).

4. CROSS-TALK BETWEEN TLR AND OTHER RECEPTORS IN RA

4.1. Cross-talk between TLR and NLRs in RA

TLRs and NLRs are two major forms of innate immune sensors for providing immediate responses against pathogenic invasion. Their activation induces the macrophages and neutrophils, and abnormalities in any of these sensor-mediated processes may lead to excessive inflammation. The NLRs are the cytoplasmic counterpart of TLRs for sensing microbial pattern and various metabolic stresses (118). Certain TLRs such as TLR2/4/9 and certain NLRs such as Nalp3 are implicated in various inflammatory diseases (67). Numerous studies have reported that NLRs complete and synergize TLRs in induction of innate immune responses and generation of IL-1β, IL-18, and IL-33. For instance, TLR ligand such as LPS induces the activation of NF-κB that is necessary for the production of pro-IL-1, and consequently pro-IL-1 is processed to IL-1 production

by NLR (Nalp3)-mediated caspase-1(67,118,122,123). Conversion of pro-IL-1 to IL-1 requires not only activation of caspase-1 which is triggered by TLR ligands and ATP, but also a second stimulus to induce the formation of the inflammasome to enhance the proteolytic maturation and secretion of IL-1β (122). Although, the induction of caspase-1 activation is independent of TLR4, secretion of mature IL-1β additionally needs TLR-dependent upregulation of IL-1β precursor and TLR-signaling is required for the assembly of a functioning inflammasome complex (122,123). A recent study reported that although blocking of IL-1 by recombinant human IL-1R antagonist (The TLRs belong to the IL-1R family (124)) has been less effective in RA compared to anti-TNF-therapy, targeting therapies to upstream signaling molecules including NLR-mediated inflammasomes might improve efficacy of current biological therapy for many autoimmune diseases (118). Therefore, attention to cross-talk between TLRs and NLRs in the pathogenesis of chronic inflammatory disorders may provide novel targets for the prevention or treatment of many common inflammatory diseases.

4.2. Cross-talk between TLR and Fc-γ receptor in RA

Although FcRs and TLRs are required for secondary antimicrobial immune responses, their co-induction may also exacerbate inflammatory diseases. Numerous studies reported cross-talk between FcRs and TLRs, and their functional profile in inflammatory immune responses. The cross-talk occurring between FcRs and the cell surface-expressed TLR1/2/4/5/6 and the endosomally expressed TLR7/8 could induce synergistic release of inflammatory cytokines after simultaneous triggering of FcγRIIA. In joints of arthritic patients, co-activation of FcRs and TLRs may contribute to the severity of the disease. Co-activation of FcγRIIA and TLR2 repolarize anti-inflammatory M2-like macrophages into M1-like macrophages with a pro-inflammatory phenotype and production of TNF-α, IL-1b, and IL-6. Attention to cross-talk between TLR and FcRs may further open up novel therapeutic options and new strategies for smart delivery systems for treatment of inflammatory diseases (125).

5. CONCLUSION AND FUTURE PROSPECT

Despite recent advances in medical therapies of RA some patients are resistant to conventional drugs and frequent and long-term use of the drugs leads to adverse events and non-specific organ toxicity. Therefore, application of drug delivery strategies based on receptor targeting as outlined herein, promises to improve patient outcome by reducing the likelihood of adverse reactions, off-target unwanted effects to conventional drugs and reducing frequency and quantity of drug administration. With greater understanding of RA, further progress on the molecular pharmacology and more in-depth research into novel carriers and drug targeted delivery to the arthritic joints can increase the possibility of successfully controlling the progression of the disease in all RA patients, and they greatly enhance the clinical treatment of RA. Recently, remarkable attentions have been paid in developing inhibitors or ligands for the specific targeting of new receptors such as TLR, FcRs, and NLR, which are involved in the TLR signaling pathways in addition to their signaling pathways. Several receptors involved in the pathological process of RA (7) are especially overexpressed in synovial tissue cells. Conventional receptors such as CD44, folate, and integrin αVβ overexpressed in synovial macrophages and fibroblasts have been widely used for active targeting in RA. Recently discovery of new receptors such as TLRs, FcRs, and NLR, many of which are expressed on synovial cells and APCs (7) not only have opened new horizons for developing new drugs but also their antagonists or humanized monoclonal antibody could be employed as ligands for targeting drug-containing carriers towards APCs and DC for the effective treatment of RA.

6. REFERENCES

1. Gabriel SE. The epidemiology of rheumatoid arthritis. Rheum Dis Clin North Am. 2001;27(2):269-281.
2. Fauci AS, Langford C. Harrison's Rheumatology. 3rd ed. New York: McGraw-Hill Education; 2013. pp. 87-106.
3. Shah A, Clair EW. Rheumatoid Arthritis. In: Jameson JL, Fauci A, Kasper D, Hauser S, Longo D, Loscalzo J. Harrison's Principles of Internal Medicine. 20th ed. New York: McGraw Hill Professional; 2018. pp. 2527-2541.
4. Ballara S, Taylor PC, Reusch P, Marmé D, Feldmann M, Maini RN, *et al*. Raised serum vascular endothelial growth factor levels are associated with destructive change in inflammatory arthritis. Arthritis Rheum. 2001;44(9):2055-2064.
5. Kumar P, Erroi A, Sattar A, Kumar S. Weibel-Palade bodies as a marker for neovascularization induced by tumor and rheumatoid angiogenesis factors. Cancer Res. 1985;45(9):4339-4348.
6. Semble E, Turner RA, McCrickard EL. Rheumatoid arthritis and osteoarthritis synovial fluid effects on primary human endothelial cell cultures. J Rheumatol. 1985;12(2):237-241.
7. Smolen JS, Steiner G. Therapeutic strategies for rheumatoid arthritis. Nat Rev Drug Discov. 2003;2(6):473-488.
8. Feng X, Chen Y. Drug delivery targets and systems for targeted treatment of rheumatoid arthritis. J Drug Target. 2018;26(10):845-857.
9. Choy EH. Clinical significance of Janus kinase inhibitor selectivity. Rheumatology (Oxford). 2019;58(6):953-962.
10. Yuan F, Quan L, Cui L, Goldring SR, Wang D. Development of macromolecular prodrug for rheumatoid arthritis. Adv Drug Deliv Rev. 2012;64(12):1205-1219.
11. Johnson BA, Haines GK, Harlow LA, Koch AE. Adhesion molecule expression in human synovial tissue. Arthritis Rheum. 1993;36(2):137-146.
12. Wilder RL. Integrin alpha V beta 3 as a target for treatment of rheumatoid arthritis and related rheumatic diseases. Ann Rheum Dis. 2002;61 (Suppl 2):ii96-ii99.
13. Nagayoshi R, Nagai T, Matsushita K, Sato K, Sunahara N, Matsuda T, *et al*. Effectiveness of anti-folate receptor beta antibody conjugated with truncated *Pseudomonas exotoxin* in the targeting of rheumatoid arthritis synovial macrophages. Arthritis Rheum. 2005;52(9):2666-2675.
14. van der Heijden JW, Oerlemans R, Dijkmans BA, Qi H, van der Laken CJ, Lems WF, *et al*. Folate receptor beta as a potential delivery route for novel folate antagonists to macrophages in the synovial tissue of rheumatoid arthritis patients. Arthritis Rheum. 2009;60(1):12-21.
15. Jamar F, Houssiau FA, Devogelaer JP, Chapman PT, Haskard DO, Beaujean V, *et al*. Scintigraphy using a technetium 99m-labelled anti-E-selectin Fab fragment in rheumatoid arthritis. Rheumatology (Oxford). 2002;41(1):53-61.
16. Kamen BA, Smith AK, Anderson RG. The folate receptor works in tandem with a probenecid-sensitive carrier in MA104 cells *in vitro*. J Clin Invest. 1991;87(4):1442-1449.
17. Antony AC. The biological chemistry of folate receptors. Blood. 1992;79(11):2807-2820.
18. Ross JF, Chaudhuri PK, Ratnam M. Differential regulation of folate receptor isoforms in normal and malignant tissues *in vivo* and in established cell lines. Physiologic and clinical implications. Cancer. 1994;73(9):2432-2443.
19. Nakashima-Matsushita N, Homma T, Yu S, Matsuda T, Sunahara N, Nakamura T, *et al*. Selective expression of folate receptor beta and its possible role in methotrexate transport in synovial macrophages from patients with rheumatoid arthritis. Arthritis Rheum. 1999;42(8):1609-1616.
20. Kinne RW, Brauer R, Stuhlmuller B, Palombo-Kinne E, Burmester GR. Macrophages in rheumatoid arthritis. Arthritis Res. 2000;2(3): 189-202.
21. Chandrasekar D, Sistla R, Ahmad FJ, Khar RK, Diwan PV. The development of folate-PAMAM dendrimer conjugates for targeted delivery of anti-arthritic drugs and their pharmacokinetics and biodistribution in arthritic rats. Biomaterials. 2007;28(3):504-512.
22. Chandrasekar D, Sistla R, Ahmad FJ, Khar RK, Diwan PV. Folate coupled poly(ethyleneglycol) conjugates of anionic poly(amidoamine) dendrimer for inflammatory tissue specific drug delivery. J Biomed Mater Res A. 2007;82(1):92-103.
23. Thomas TP, Goonewardena SN, Majoros IJ, Kotlyar A, Cao Z, Leroueil PR, *et al*. Folate-targeted nanoparticles show efficacy in the treatment of inflammatory arthritis. Arthritis Rheum. 2011;63(9):2671-2680.
24. Mansouri S, Cuie Y, Winnik F, Shi Q, Lavigne P, Benderdour M, *et al*. Characterization of folate-chitosan-DNA nanoparticles for gene therapy. Biomaterials. 2006;27(9):2060-2065.
25. Hattori Y, Sakaguchi M, Maitani Y. Folate-linked lipid-based nanoparticles deliver a NFkappaB decoy into activated murine macrophage-like RAW264.7 cells. Biol Pharm Bull. 2006;29(7):1516-1520.
26. Duan W, Li H. Combination of NF-kB targeted siRNA and methotrexate in a hybrid nanocarrier towards the effective treatment in rheumatoid arthritis. J Nanobiotechnol. 2018;16(1):58-66.
27. Sun X, Dong S, Li X, yu K, Sun F, Lee R, *et al*. Delivery of siRNA using folate receptor-targeted pH-sensitive polymeric nanoparticles for rheumatoid arthritis therapy. Nanomed Nanotechnol. 2019; 20:1-11.
28. Zhao J, Zhang X, Sun X, Zhao M, Yu C, Lee RJ, *et al*. Dual-functional lipid polymeric hybrid pH-responsive nanoparticles decorated with cell penetrating peptide and folate for therapy against rheumatoid arthritis. Eur J Pharm Biopharm. 2018;130:39-47.

29. Zhang N, Xu C, Li N, Zhang S, Fu L, Chu X, *et al*. Folate receptor-targeted mixed polysialic acid micelles for combating rheumatoid arthritis: *in vitro* and *in vivo* evaluation. Drug Deliv. 2018;25(1):1182-1191.
30. Verma A, Jain A, Tiwari A, Saraf S, Panda PK, Agrawal GP, *et al*. Folate conjugated double liposomes bearing prednisolone and methotrexate for targeting rheumatoid arthritis. Pharm Res. 2019;36(8):123-135.
31. Fischer C, Thiele HG, Hamann A. Adhesion molecules and homing in inflamed synovial. Immun Infekt. 1993;1:12-3.
32. Haynes BF, Hale LP, Patton KL, Martin ME, McCallum RM. Measurement of an adhesion molecule as an indicator of inflammatory disease activity. Up-regulation of the receptor for hyaluronate (CD44) in rheumatoid arthritis. Arthritis Rheum. 1991;34(11):1434-1443.
33. Naor D, Nedvetzki S. CD44 in rheumatoid arthritis. Arthritis Res Ther. 2003;5(3):105-115.
34. Heo R, Park JS, Jang HJ, Kim SH, Shin JM, Suh YD, *et al*. Hyaluronan nanoparticles bearing gamma-secretase inhibitor: *in vivo* therapeutic effects on rheumatoid arthritis. J Control Release. 2014;192:295-300.
35. Yu C, Li X, Hou Y, Meng X, Wang D, Liu J, *et al*. Hyaluronic acid coated acid-sensitive nanoparticles for targeted therapy of adjuvant-induced arthritis in rats. Molecules. 2019;24(1):E146-E161.
36. Zhou M, Hou J, Zhong Z, Hao N, Lin Y, Li C. Targeted delivery of hyaluronic acid-coated solid lipid nanoparticles for rheumatoid arthritis therapy. Drug Deliv. 2018;25(1):716-722.
37. Lee H, Lee MY, Bhang SH, Kim BS, Kim YS, Ju JH, *et al*. Hyaluronate-gold nanoparticle/tocilizumab complex for the treatment of rheumatoid arthritis. ACS Nano. 2014;8(5):4790-4798.
38. Shin JM, Kim SH, Thambi T, You DG, Jeon J, Lee JO, *et al*. A hyaluronic acid-methotrexate conjugate for targeted therapy of rheumatoid arthritis. Chem Commun (Camb). 2014;50(57):7632-7635.
39. Kim YJ, Chae SY, Jin CH, Sivasubramanian M, Son S, Choi KY, *et al*. Ionic complex systems based on hyaluronic acid and PEGylated TNF-related apoptosis-inducing ligand for treatment of rheumatoid arthritis. Biomaterials. 2010;31(34):9057-9064.
40. Tamura T, Higuchi Y, Kitamura H, Murao N, Saitoh R, Morikawa T, *et al*. Novel hyaluronic acid-methotrexate conjugate suppresses joint inflammation in the rat knee: efficacy and safety evaluation in two rat arthritis models. Arthritis Res Ther. 2016;18:79-89.
41. Jeon J, Rao NV, Byun JH, Heo R, Han HS, Park JH. pH-Responsive hyaluronic acid-based nanocarrier for treatment of rheumatoid arthritis. J Nanosci Nanotechnol. 2016;16(11):11849-11856.
42. Seo J, Park SH, Kim MJ, Ju HJ, Yin XY, Min BH, *et al*. Injectable click-crosslinked hyaluronic acid depot to prolong therapeutic activity in articular joints affected by rheumatoid arthritis. ACS Appl Mater Interfaces. 2019;11(28):24984-24998.
43. Jung YS, Park W, Park H, Lee DK, Na K. Thermo-sensitive injectable hydrogel based on the physical mixing of hyaluronic acid and Pluronic F-127 for sustained NSAID delivery. Carbohyd Polym. 2017;156:403-408.
44. Gouveia VM, Lopes-de-Araujo J, Costa Lima SA, Nunes C, Reis S. Hyaluronic acid-conjugated pH-sensitive liposomes for targeted delivery of prednisolone on rheumatoid arthritis therapy. Nanomedicine (Lond). 2018;13(9):1037-1049.
45. Hsu AR, Veeravagu A, Cai W, Hou LC, Tse V, Chen X. Integrin alpha v beta 3 antagonists for anti-angiogenic cancer treatment. Recent Pat Anticancer Drug Discov. 2007;2(2):143-158.
46. Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin alpha v beta 3 for angiogenesis. Science. 1994;264(5158):569-571.
47. Tsou R, Isik FF. Integrin activation is required for VEGF and FGF receptor protein presence on human microvascular endothelial cells. Mol Cell Biochem. 2001;224(1-2):81-89.
48. Weber AJ, De Bandt M, Gaudry M. Immunohistochemical analysis of vascular endothelial growth factor expression in severe and destructive rheumatoid arthritis. J Rheumatol. 2000;27(9):2284-2286.
49. De Bandt M, Mahdi MHB, Ollivier V, Grossin M, Dupuis M, Gaudry M, *et al*. Blockade of vascular endothelial growth factor receptor I (VEGF-RI), but not VEGF-RII, suppresses joint destruction in the K/BxN model of rheumatoid arthritis. J Immunol. 2003;171(9):4853-4859.
50. Lainer-Carr D, Brahn E. Angiogenesis inhibition as a therapeutic approach for inflammatory synovitis. Nat Clin Pract Rheumatol. 2007;3(8):434-442.
51. Yumoto K, Ishijima M, Rittling SR, Tsuji K, Tsuchiya Y, Kon S, *et al*. Osteopontin deficiency protects joints against destruction in anti-type II collagen antibody-induced arthritis in mice. Proc Natl Acad Sci USA. 2002;99(7):4556-4561.
52. Scheinman RI, Trivedi R, Vermillion S, Kompella UB. Functionalized STAT1 siRNA nanoparticles regress rheumatoid arthritis in a mouse model. Nanomedicine (Lond). 2011;6(10):1669-1682.
53. Lee SM, Kim HJ, Ha YJ, Park YN, Lee SK, Park YB, *et al*. Targeted chemo-photothermal treatments of rheumatoid arthritis using gold half-shell multifunctional nanoparticles. ACS Nano. 2013;7(1):50-57.
54. Koning GA, Schiffelers RM, Wauben MH, Kok RJ, Mastrobattista E, Molema G, *et al*. Targeting of angiogenic endothelial cells at sites of inflammation by dexamethasone phosphate-containing RGD peptide liposomes inhibits experimental arthritis. Arthritis Rheum. 2006;54(4):1198-1208.
55. Wang Y, Liu Z, Li T, Chen L, Lyu J, Li C, *et al*. Enhanced therapeutic effect of RGD-modified polymeric micelles loaded with low-dose methotrexate and nimesulide on Rheumatoid Arthritis. Theranostics. 2019;9(3):708-720.

56. Ye C, Kiriyama K, Mistuoka C, Kannagi R, Ito K, Watanabe T, *et al*. Expression of E-selectin on endothelial cells of small veins in human colorectal cancer. Int J Cancer. 1995;61(4):455-460.
57. Vallien G, Langley R, Jennings S, Specian R, Granger DN. Expression of endothelial cell adhesion molecules in neovascularized tissue. Microcirculation. 2000;7(4):249-258.
58. Bhaskar V, Law DA, Ibsen E, Breinberg D, Cass KM, DuBridge RB, *et al*. E-selectin up-regulation allows for targeted drug delivery in prostate cancer. Cancer Res. 2003;63(19): 6387-6394.
59. Ehrhardt C, Kneuer C, Bakowsky U. Selectins-an emerging target for drug delivery. Adv Drug Deliv Rev. 2004;56(4):527-549.
60. Magnani JL. The discovery, biology, and drug development of sialyl Lea and sialyl Lex. Arch Biochem Biophys. 2004;426(2):122-131.
61. Simanek EE, McGarvey GJ, Jablonowski JA, Wong CH. Selectin-carbohydrate interactions: from natural ligands to designed mimics. Chem Rev. 1998;98(2):833-862.
62. Kaila N, Thomas BE 4th. Design and synthesis of sialyl Lewis(x) mimics as E- and P-selectin inhibitors. Med Res Rev. 2002;22(6):566-601.
63. Everts M, Koning GA, Kok RJ, Asgeirsdottir SA, Vestweber D, Meijer DK, *et al*. *In vitro* cellular handling and *in vivo* targeting of E-selectin-directed immunoconjugates and immunoliposomes used for drug delivery to inflamed endothelium. Pharm Res. 2003;20(1):64-72.
64. Mitragotri S, Yoo JW. Designing micro- and nano-particles for treating rheumatoid arthritis. Arch Pharm Res. 2011;34(11):1887-1897.
65. Hirai M, Minematsu H, Kondo N, Oie K, Igarashi K, Yamazaki N. Accumulation of liposome with Sialyl Lewis X to inflammation and tumor region: application to *in vivo* bio-imaging. Biochem Biophys Res Commun. 2007;353(3):553-558.
66. Patra MC, Choi S. Recent progress in the development of toll-like receptor (TLR) antagonists. Expert Opin Ther Pat. 2016;26(6):719-730.
67. McCormack WJ, Parker AE, O'Neill LA. Toll-like receptors and NOD-like receptors in rheumatic diseases. Arthritis Res Ther. 2009;11(5):243-250.
68. Mayadas TN, Tsokos GC, Tsuboi N. Mechanisms of immune complex-mediated neutrophil recruitment and tissue injury. Circulation. 2009;120(20):2012-2024.
69. Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. Nat Rev Immunol. 2008;8(1):34-47.
70. Silman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. Arthritis Res. 2002;4(Suppl 3):S265-S272.
71. Andreakos E, Sacre S, Foxwell BM, Feldmann M. The toll-like receptor-nuclear factor kappaB pathway in rheumatoid arthritis. Front Biosci. 2005;10:2478-2488.
72. Imler JL, Hoffmann JA. Toll receptors in innate immunity. Trends Cell Biol. 2001;11(7):304-311.
73. Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immuno. 2003;21(1):335-376.
74. Kempsell KE, Cox CJ, Hurle M, Wong A, Wilkie S, Zanders ED, *et al*. Reverse transcriptase-PCR analysis of bacterial rRNA for detection and characterization of bacterial species in arthritis synovial tissue. Infect Immun. 2000;68(10): 6012-6026.
75. van der Heijden IM, Wilbrink B, Tchetverikov I, Schrijver IA, Schouls LM, Hazenberg MP, *et al*. Presence of bacterial DNA and bacterial peptidoglycans in joints of patients with rheumatoid arthritis and other arthritides. Arthritis Rheum. 2000;43(3):593-598.
76. Roelofs MF, Joosten LA, Abdollahi-Roodsaz S, van Lieshout AW, Sprong T, van den Hoogen FH, *et al*. The expression of toll-like receptors 3 and 7 in rheumatoid arthritis synovium is increased and costimulation of toll-like receptors 3, 4, and 7/8 results in synergistic cytokine production by dendritic cells. Arthritis Rheum. 2005;52(8):2313-2322.
77. Iwahashi M, Yamamura M, Aita T, Okamoto A, Ueno A, Ogawa N, *et al*. Expression of toll-like receptor 2 on CD16+ blood monocytes and synovial tissue macrophages in rheumatoid arthritis. Arthritis Rheum. 2004;50(5):1457-1467.
78. Murata M. Activation of Toll-like receptor 2 by a novel preparation of cell wall skeleton from *Mycobacterium bovis* BCG Tokyo (SMP-105) sufficiently enhances immune responses against tumors. Cancer Sci. 2008;99(7):1435-1440.
79. Ultaigh SN, Saber TP, McCormick J, Connolly M, Dellacasagrande J, Keogh B, *et al*. Blockade of Toll-like receptor 2 prevents spontaneous cytokine release from rheumatoid arthritis *ex vivo* synovial explant cultures. Arthritis Res Ther. 2011;13(1):R33-R41.
80. Elshabrawy HA, Essani AE, Szekanecz Z, Fox DA, Shahrara S. TLRs, future potential therapeutic targets for RA. Autoimmun Rev. 2017;16(2): 103-113.
81. Wu YW, Tang W, Zuo JP. Toll-like receptors: potential targets for lupus treatment. Acta Pharmacol Sin. 2015;36(12):1395-1407.
82. Thwaites R, Chamberlain G, Sacre S. Emerging role of endosomal toll-like receptors in rheumatoid arthritis. Front Immunol. 2014;5(1):1-8.
83. Muzio M, Bosisio D, Polentarutti N, D'amico G, Stoppacciaro A, Mancinelli R, *et al*. Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. J Immunol. 2000;164(11):5998-6004.
84. Brentano F, Schorr O, Gay RE, Gay S, Kyburz D. RNA released from necrotic synovial fluid cells activates rheumatoid arthritis synovial fibroblasts *via* Toll-like receptor 3. Arthritis Rheum. 2005;52(9):2656-2665.
85. Paulsen F, Pufe T, Conradi L, Varoga D, Tsokos M, Papendieck J, *et al*. Antimicrobial peptides are expressed and produced in healthy and inflamed

human synovial membranes. J Pathol. 2002;198(3):369-377.
86. Lai Y, Adhikarakunnathu S, Bhardwaj K, Ranjith-Kumar C, Wen Y, Jordan JL, *et al*. LL37 and cationic peptides enhance TLR3 signaling by viral double-stranded RNAs. PLoS One. 2011;6(10):e26632.
87. Hu F, Mu R, Zhu J, Shi L, Li Y, Liu X, *et al*. Hypoxia and hypoxia-inducible factor-1α provoke toll-like receptor signalling-induced inflammation in rheumatoid arthritis. Ann Rheum Dis. 2014;73(5):928-936.
88. Moon SJ, Park MK, Oh HJ, Lee SY, Kwok SK, Cho ML, *et al*. Engagement of toll-like receptor 3 induces vascular endothelial growth factor and interleukin-8 in human rheumatoid synovial fibroblasts. Korean J Intern Med. 2010;25(4): 429-435.
89. Meng L, Zhu W, Jiang C, He X, Hou W, Zheng F, *et al*. Toll-like receptor 3 upregulation in macrophages participates in the initiation and maintenance of pristane-induced arthritis in rats. Arthritis Res Ther. 2010;12(3):R103-R114.
90. Zhu W, Meng L, Jiang C, Xu J, Wang B, Han Y, *et al*. Overexpression of toll-like receptor 3 in spleen is associated with experimental arthritis in rats. Scand J Immunol. 2012;76(3):263-270.
91. O'Neill LA, Bryant CE, Doyle SL. Therapeutic targeting of toll-like receptors for infectious and inflammatory diseases and cancer. Pharmacol Rev. 2009;61(2):177-197.
92. Roelofs MF, Boelens WC, Joosten LA, Abdollahi-Roodsaz S, Geurts J, Wunderink LU, *et al*. Identification of small heat shock protein B8 (HSP22) as a novel TLR4 ligand and potential involvement in the pathogenesis of rheumatoid arthritis. J Immunol. 2006;176(11):7021-7027.
93. Midwood K, Sacre S, Piccinini AM, Inglis J, Trebaul A, Chan E, *et al*. Tenascin-C is an endogenous activator of toll-like receptor 4 that is essential for maintaining inflammation in arthritic joint disease. Nat Med. 2009;15(7):774-780.
94. Pierer M, Wagner U, Rossol M, Ibrahim S. Toll-like receptor 4 is involved in inflammatory and joint destructive pathways in collagen-induced arthritis in DBA1J mice. PLoS One. 2011;6(8):e23539.
95. Campo GM, Avenoso A, Campo S, D'Ascola A, Nastasi G, Calatroni A. Molecular size hyaluronan differently modulates toll-like receptor-4 in LPS-induced inflammation in mouse chondrocytes. Biochimie. 2010;92(2):204-215.
96. Campo GM, Avenoso A, D'Ascola A, Scuruchi M, Prestipino V, Nastasi G, *et al*. The inhibition of hyaluronan degradation reduced pro-inflammatory cytokines in mouse synovial fibroblasts subjected to collagen-induced arthritis. J Cell Biochem. 2012;113(6):1852-1867.
97. Noble PW, McKee CM, Cowman M, Shin HS. Hyaluronan fragments activate an NF-kappa B/I-kappa B alpha autoregulatory loop in murine macrophages. J Exp Med. 1996;183(5):2373-2378.
98. Termeer CC, Hennies J, Voith U, Ahrens T, Weiss JM, Prehm P, *et al*. Oligosaccharides of hyaluronan are potent activators of dendritic cells. J Immunol. 2000;165(4):1863-1870.
99. Campo GM, Avenoso A, D'Ascola A, Prestipino V, Scuruchi M, Nastasi G, *et al*. Hyaluronan differently modulates TLR-4 and the inflammatory response in mouse chondrocytes. Biofactors. 2012;38(1):69-76.
100. Monnet E, Shang L, Lapeyre G, deGraaf K, Hatterer E, Buatois V, *et al*. AB0451 NI-0101, a monoclonal antibody targeting toll like receptor 4 (TLR4) being developed for rheumatoid arthritis (RA) treatment with a potential for personalized medicine. Ann Rheum Dis. 2015;74(2):1046.
101. Park S, Shin HJ, Shah M, Cho HY, Anwar MA, Achek A, *et al*. TLR4/MD2 specific peptides stalled in vivo LPS-induced immune exacerbation. Biomaterials. 2017;126:49-60.
102. Babazada H, Yamashita F, Hashida M. Suppression of experimental arthritis with self-assembling glycol-split heparin nanoparticles *via* inhibition of TLR4-NF-kappaB signaling. J Control Release. 2014;194:295-300.
103. Rao K, Roome T, Aziz S, Razzak A, Abbas G, Imran M, *et al*. Bergenin loaded gum xanthan stabilized silver nanoparticles suppress synovial inflammation through modulation of the immune response and oxidative stress in adjuvant induced arthritic rats. J Mater Chem B. 2018;6(27): 4486-4501.
104. Roome T, Aziz S, Razzak A, Aslam Z, Lubna, Jamali KS, *et al*. Opuntioside, opuntiol and its metallic nanoparticles attenuate adjuvant-induced arthritis: novel suppressors of toll-like receptors -2 and -4. Biomed Pharmacother. 2019;112:1-15.
105. Chamberlain ND, Vila OM, Volin MV, Volkov S, Pope RM, Swedler W, *et al*. TLR5, a novel and unidentified inflammatory mediator in rheumatoid arthritis that correlates with disease activity score and joint TNF-α levels. J Immunol. 2012;189(1):475-483.
106. Alzabin S, Kong P, Medghalchi M, Palfreeman A, Williams R, Sacre S. Investigation of the role of endosomal toll-like receptors in murine collagen-induced arthritis reveals a potential role for TLR7 in disease maintenance. Arthritis Res Ther. 2012;14(3):R142-R152.
107. Alzabin S, Williams RO. Effector T cells in rheumatoid arthritis: lessons from animal models. FEBS Lett. 2011;585(23):3649-3659.
108. Hayashi T, Gray CS, Chan M, Tawatao RI, Ronacher L, McGargill MA, *et al*. Prevention of autoimmune disease by induction of tolerance to Toll-like receptor 7. Proc Natl Acad Sci USA. 2009;106(8):2764-2769.
109. Demaria O, Pagni PP, Traub S, de Gassart A, Branzk N, Murphy AJ, *et al*. TLR8 deficiency leads to autoimmunity in mice. J Clin Invest. 2010;120(10):3651-3662.
110. Li Y, Berke IC, Modis Y. DNA binding to proteolytically activated TLR9 is sequence-

independent and enhanced by DNA curvature. EMBO J. 2012;31(4):919-931.
111. Viglianti GA, Lau CM, Hanley TM, Miko BA, Shlomchik MJ, Marshak-Rothstein A. Activation of autoreactive B cells by CpG dsDNA. Immunity. 2003;19(6):837-847.
112. Fischer A, Abdollahi-Roodsaz S, Bohm C, Niederreiter B, Meyer B, Yau ACY, *et al*. The involvement of Toll-like receptor 9 in the pathogenesis of erosive autoimmune arthritis. J Cell Mol Med. 2018;22(9):4399-4409.
113. Torigoe M, Sakata K, Ishii A, Iwata S, Nakayamada S, Tanaka Y. Hydroxychloroquine efficiently suppresses inflammatory responses of human class-switched memory B cells *via* Toll-like receptor 9 inhibition. Clin Immunol. 2018;195:1-7.
114. Sacre S, Medghalchi M, Gregory B, Brennan F, Williams R. Fluoxetine and citalopram exhibit potent antiinflammatory activity in human and murine models of rheumatoid arthritis and inhibit toll-like receptors. Arthritis Rheum. 2010;62(3):683-693.
115. Kandimalla ER, Bhagat L, Wang D, Yu D, Sullivan T, La Monica N, *et al*. Design, synthesis and biological evaluation of novel antagonist compounds of toll-like receptors 7, 8 and 9. Nucleic Acids Res. 2013;41(6):3947-3961.
116. Tsai CY, Lu SL, Hu CW, Yeh CS, Lee GB, Lei HY. Size-dependent attenuation of TLR9 signaling by gold nanoparticles in macrophages. J Immunol. 2012;188(1):68-76.
117. Peng B, Liang H, Li Y, Dong C, Shen J, Mao HQ, *et al*. Tuned cationic dendronized polymer: molecular scavenger for rheumatoid arthritis treatment. Angew Chem. 2019;131(13):4298-4302.
118. Fukata M, Vamadevan AS, Abreu MT. Toll-like receptors (TLRs) and Nod-like receptors (NLRs) in inflammatory disorders. Semin Immunol. 2009;21(4):242-253.
119. Guo C, Fu R, Wang S, Huang Y, Li X, Zhou M, *et al*. NLRP3 inflammasome activation contributes to the pathogenesis of rheumatoid arthritis. Clin Exp Immunol. 2018;194(2):231-243.
120. Yang Y, Zhang X, Xu M, Wu X, Zhao F, Zhao C. Quercetin attenuates collagen-induced arthritis by restoration of Th17/Treg balance and activation of heme oxygenase 1-mediated anti-inflammatory effect. Int Immunopharmacol. 2018;54:153-162.
121. Chen B, Vousden KA, Naiman B, Turman S, Sun H, Wang S, *et al*. Humanised effector-null FcγRIIA antibody inhibits immune complex-mediated proinflammatory responses. Ann Rheum Dis. 2019;78(2):228-237.
122. Church LD, Cook GP, McDermott MF. Primer: inflammasomes and interleukin 1beta in inflammatory disorders. Nat Clin Pract Rheumatol. 2008;4(1):34-42.
123. Kanneganti TD, Lamkanfi M, Kim YG, Chen G, Park JH, Franchi L, *et al*. Pannexin-1-mediated recognition of bacterial molecules activates the cryopyrin inflammasome independent of toll-like receptor signaling. Immunity. 2007;26(4):433-443.
124. Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb Perspect Biol. 2009;1(6):a001651,1-10.
125. van Egmond M, Vidarsson G, Bakema JE. Cross-talk between pathogen recognizing Toll-like receptors and immunoglobulin Fc receptors in immunity. Immunol Rev. 2015;268(1):311-327.