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REVIEW ARTICLE

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Research progress on serological indices and their clinical application in rheumatoid arthritis

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Abstract

Background: As a chronic systemic autoimmune disease of undetermined etiology, rheumatoid arthritis (RA) has a complex pathogenesis, which involves multiple proteins and cytokines. The 2010 ACR/EULAR classification criteria facilitate early diagnosis of RA with reduced specificity when compared to the 1987 ACR criteria. Hence, it is imperative to identify novel serological inflammatory indicators and targets, in order to explain the complex regulatory network of RA. The present review discusses the associations of various inflammatory factors with RA and its underlying mechanism. Besides, the review also provides a novel insight into the clinical treatment of RA.

Materials and Methods: According to the PRISMA guidelines, databases like Web of Science, Google-Scholar, Pubmed and Scopus were systematically searched for articles from January 1, 2018 to January 1, 2022 using The following 2 keywords: "rheumatoid arthritis", "Inflammatory cytokines", "ILs", "serum amyloid protein A", "matrix metalloproteinase 3", "RANKL", "Glucose-6-phosphoisomerase", "Anti-keratin antibody", "1,25-Dihydroxyvitamin D3".

Results: Indicators like MMPs, ILs, glucose-6-phosphate isomerase (GPI), anti-keratin antibody (AKA) and receptor activator of nuclear factor-κB ligand (RANKL) are the current hotspots in the efficacy research of RA. The present review suggests that ILs are highly expressed in the serum and synovial tissues of RA patients. By targeted inhibition of ILs with inhibitor application, precise RA treatment can be achieved.

Conclusions: Based on these results, it can be concluded that inflammatory factors have certain guiding significance in the diagnosis and efficacy evaluation of RA. However, the mechanisms of interactions among them are rather complex, which deserve further exploration.

KEYWORDS
interleukin, matrix metalloproteinase 3, overview, rheumatoid arthritis, serum amyloid protein a

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1 | INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune syndrome characterized by nonspecific and symmetrical peripheral joint inflammation; chronic inflammation; synovial membrane hyperplasia; vascular opacity formation; and invasion of articular cartilage, subchondral bone, ligaments, and tendons, which result in the destruction of the articular cartilage, bone, and joint capsule, eventually leading to joint deformity and varying degrees of systemic manifestations.[1]

RA is a common and highly debilitating disease, with a female predilection and prevalence of 0.32%–0.38% in China. The disease is observed frequently in the 30-50-year age group. Bone and joint destruction may occur within half a year, whereas the short treatment period refers to the period within 2 years of disease onset. Therefore, the search for highly sensitive and specific serological indicators is of great clinical significance for the early diagnosis, treatment, and monitoring of RA.

2 | CLASSIC RA INDICATORS COMMONLY USED IN CLINICAL DIAGNOSIS

2.1 | Anti-streptococcus O

Antistreptolysin O (ASO) is an antibody targeted against streptolysin O, a toxic enzyme produced by group A streptococcus bacteria, which lyses red blood cells and exerts a toxic effect on numerous cells in the body. High ASO levels can be observed in the serum of humans infected with *Streptococcus haemolyticus*. Thus, ASO detection can facilitate the diagnosis of metaplastic disease following streptococcal infection. ASO values can be normal, elevated, or decreased in the serotype RA classification. Elevated ASO levels do not necessarily indicate RA but rather a recent streptococcal infestation. Thus, ASO detection has not been recommended as a routine test for RA.

2.2 | Anticyclopanine peptide antibody

Anticyclopanine peptide antibody (ACCP) is a peptide fragment of cyclic polyserine that comprises 20 amino acids with a predominant IgG profile; it appears early in the disease course. In 2000, Schellkens et al[2] synthesized CCP as an antigen for the enzyme-linked immunosorbent assay, with high specificity as a serological marker for the early diagnosis of RA and with no sex or age correlation. ACCP has been verified to predict RA severity. Patients with RA and positive ACCP exhibit more severe bone and joint destruction than those with negative ACCP. However, its diagnostic sensitivity is slightly low. ACCP may be used in combination with rheumatoid factor (RF), extractable nuclear antigen (ENA), and antinuclear antibody (ANA) to improve the sensitivity and specificity of the diagnosis.

2.3 | Rheumatoid factor

RF is an autoantibody that targets the degenerated globulin IgG Fc segment and is the first autoantibody identified in RA. RF is not species-specific and includes three types of antibodies, namely IgM, IgG, and IgA, with IgM being used in the diagnosis of various rheumatic diseases. RF causes inflammatory tissue damage, and it involves inflammatory factors and inflammatory adhesion molecules, which can lead to osteoarthritis and vasculitis. RF sensitivity was low at 82.34% for early diagnosis, with other indicators being required. RF is not specific due to its expression in both connective tissue and non-connective tissue diseases such as diffuse pulmonary fibrosis, cirrhosis, nodular disease, systemic lupus erythematosus, and tumors. Some healthy individuals can also be positive but with low titers.[3] RF serum levels in patients with RA are significantly higher than those in other groups, which is crucial for RA diagnosis. Increased RF titers indicate active disease and progressive joint destruction, whereas decreasing titers indicate improvement and secondary changes following remission. The commonly used diagnostic method is the latex particle agglutination test with IgG adsorption and turbidimetry (normal value: 20 IU/mL). This method has low sensitivity and specificity and can only detect serum IgM-RF.

2.4 | Immunoglobulin and its complement

IgG, which is the most abundant immunoglobulin in the serum and the main antibody of the re-immune response, is involved in the local mucosal immune response, whereas IgM is the first antibody to be synthesized and secreted by the human body. The invasion of pathogenic factors leads to the production of various cytokines by Th cells. The activated B cells produce several antibodies, which combine with antigens to form a complex that is deposited on the synovial membrane to activate complement, thus eliciting an immune response. Serum immunoglobulin levels are higher in patients with RA than in healthy subjects. Additionally, these levels are higher in active disease than in remission, with the level of change correlating with the degree of disease activity. The complement is a type of glycoprotein in humans and vertebrate serum with enzyme activity and thermostability. The complement system is crucial for the maintenance of immune homeostasis including RA pathogenesis. The complement levels in patients with RA remain controversial, with C3 and C4 levels generally elevated. However, some studies have exhibited that C3 and C4 levels are reduced.[4]

2.5 | ANA and ENA

ANA refers to the antibodies produced against nuclear components and is the general term for a group of autoantibodies whose target antigens include various components of eukaryotic cells such as DNP, DNA, ENA, and RNA. ANA can react with the nuclei of all animals and mainly exists in serum, pleural effusion, and synovial fluid. ANA is not organ- or genus-specific and can be observed in numerous

autoimmune diseases. The ANA detection rate is approximately 60% in patients with RA, whereas the ENA detection rate is relatively low. Although this parameter can be used as a preliminary screening index, it is not recommended as a specific index. Multiple indices must be combined to avoid missed diagnosis and misdiagnosis.

3 | RECENT RESEARCH PROGRESS ON DIAGNOSTIC INDICATORS FOR RA

In addition to the classical immunological tests, more immunological indicators are being discovered and applied in RA diagnosis as RA mechanisms are being intensively explored. Details are presented in Table 1 and Table 2. Table 1 shows the inflammatory factors produced by different types of cells and the regulation on them by different treatment methods. Table 2 illustrates the interactions between inflammatory factors and their effects on whole body and synovial/bone levels.

3.1 | Cytokines

3.1.1 | Interleukin 6

Interleukin 6 (IL-6) is a glycoprotein with 184 amino acids and is derived from activated monocytes and macrophages. It induces the secretion of acute proteins from the hepatocytes and the release of chemokines from the endothelial cells, which are vital for the acute immune and hematopoietic responses. IL-6 acts through the liver, which induces the production of C-reactive protein (CRP), SAA, Hp, and FIB, stimulates RANKL from osteoblasts, and activates osteoclasts. The high IL-6 levels stimulate the synthesis of immunoglobulins and RFs from B cells, induce the production and release of cytokines (IL-2, TNF-α), and enhance the inflammatory effects.[5] IL-6 acts on the osteogenic precursor cells to regulate the transcription of MAPK, phosphatase, and ubiquitin pathway-related genes; inhibit precursor cell differentiation; and induce the production of MMP-3 and MMP-13 by synovial cells, causing joint, and cartilage destruction. Additionally, IL-6 exerts biological activity on the target cells through IL-6R, and the transmembrane protein gp130. gp130 dimerization leads to JAK signaling and transcriptional activation of STAT3, triggering intracellular cascades. Clinical studies have exhibited the positive correlation between elevated levels of IL-6 and RF, CRP, duration of morning stiffness, Ritchie Joint Index, and disease activity.

Studies have indicated the efficacy and tolerance of the novel IL-6 antagonist tocilizumab (TCZ), either alone or in combination with anti-rheumatic drugs, in patients exhibiting poor outcomes with disease-modifying anti-rheumatic drugs (DMARDs).[6,7]

3.1.2 | Interleukin 15

Interleukin 15 (IL-15) is a member of the 4a-helical cytokine family, with a molecular weight of 14–15 kD. It is homologous to IL-2 and is involved in RA pathogenesis and differentiation and proliferation of T cells and NK cells. IL-15 mRNA can be expressed on the surface of various cells (such as activated monocytes, epithelial cells, and astrocytes) and tissues (such as placenta, skeletal muscle, heart, lung, liver, and kidney).[8] IL-15Rα-knockout mice exhibit lymphocyte proliferation and impaired homing, particularly $CD8^+$ subpopulation, which suppress apoptosis. Similar to IL-2, the IL-15Rα/β/γ complex regulates JAK1/3 and STAT3/5 through signaling. Recruited and activated T cells enter the synovial membrane and induce synovial-derived macrophages to synthesize TNF, which is involved in RA pathogenesis. A cascade response is generated that promotes the production of TNF-α, TNF-β, IL-6, IL-8, monocyte chemotactic protein (MCP-1), IL-17, and MMP-3, inflammatory cell aggregation, and amplification of the inflammatory response.[9] Simultaneous negative feedback regulation of TNF-α in synovial fluid stimulates IL-15 production, constituting a large positive and negative feedback regulatory pathway.[10] Studies have indicated that IL-15 plays a role in the early stage of RA, with elevated IL-15 values predicting disease severity, which positively correlates with the duration of morning stiffness, Ritchie index, disease activity score (DAS), HAQ, ESR, CRP, and RF, and negatively correlates with grip strength. Thus, it can be used as an independent RA biomarker.[11] IL-15 induces polyclonal B cells to produce IgM, IgA, and IgG, which are the crucial targets for RA treatment with satisfactory efficacy.[12] AMG 714 is an anti-human Ig G1-κ monoclonal antibody that blocks IL-15 and is effective in clinical trials.

TABLE 1 List of cytokines

Interleukin family	Source	Mechanism of action	Clinical application
1. IL-6	Activated monocytes and macrophages	IL-6, gp130, Jak, pI3K, AKt, mTOR, Stat3	Widely used, for example, tocilizumab
2. IL-15	Activated monocytes, epithelial cells, astroglial microglia, etc.	IL-15R/α/β/γ, JAK, pI3K, RAS, RAF, MEK, ERK	AMG714
3. IL-17	T lymphocytes	IL-17R, ACT1, TRAF6, MAPK, AP-1	Secukinumab (AIN457)
4. IL-23	Dendritic cells, phagocytes, and microglia	IL-23R, JAK2, STAT3	Ustekinumab Guselkumab
5. IL-38	Basal layer cells and proliferating B cells	IL-36R, IL-1RAcP, MyD88, NF-κB, MAPK	Not found yet.

TABLE 2 List of cytokines

Cytokines	Interactions between inflammatory factors	Effects on whole body and synovial/bone level
IL-6	a. Positive correlation with TJC, SJC, VAS, PtGA, PGA, CRP, ESR, and DAS28; b. Positive correlation with IL-17 in the serum of patients with RA; c. Inhibition of LPS-induced IL-6 by IL-38 (20–152); d. Upregulation of SAA expression in the acute phase of RA with IL-6 release; e. Inhibition of MMP3 overexpression in rat synovial tissue with reduced IL-6 secretion; f. IL-6/sIL-6R induces osteoclast differentiation in response to low concentrations (10 ng/mL) of RANKL and inhibits osteoclast differentiation in response to high concentrations (50 ng/mL) of RANKL; g. The IL-6/IL-17 axis plays an important role in GPI-induced arthritis;	IL-6 was significantly higher in serum and synovial fluid of patients with RA than in those of controls
IL-15	a. Positive correlation with time to morning stiffness, Ritchie index, HAQ, ESR, CRP, and RF; b. Synergistic interaction with IL-17; c. Upregulation of RANKL expression, especially in CD28-T cells, and increased osteoclast formation with IL-15 stimulation;	High levels of IL-15 and IL-15 mRNA detected in the synovial membrane, synovial fluid, and serum of RA patients
IL-17	a. Positive correlations with age, TJC, VAS, PtGA, PGA, ESR, CRP, RF, DAS28, CCP, and joint function grading; b. Promoting the production of IL-17; c. Negative correlation with IL-38 in RA; d. SAA induces neutrophils at the site of inflammation and induces IL-17 secretion; e. Promotion of MMP3 production; f. Promotion of RANKL expression by osteoblasts; g. No significant correlation with GPI; h. Negative correlation with 25(OH)D in patients with RA; i. Negative correlation with IL-10 in patients with RA;	IL-17 was significantly elevated in RA sera
IL-23	a. Positive correlation with time to morning stiffness, VAS, joint pressure pain index, joint swelling index, PLT, CRP, and bone destruction score; b. SAA is an important inducer of IL-23 in synoviocytes and activates the IL-23/IL-17 pathway; c. IL-23p19 significantly promotes RA-FLS RANKL expression; d. 25(OH)D negatively correlates with IL-23 in patients with RA;	High expression of IL-23p19 in RA synovium
IL-38	a. Negative correlation with CRP, IL-6, IL-1Rα, and leptin; b. Reduced IL-23 expression levels; c. Upregulated OPG expression and downregulated RANKL expression in CIA rats.	Increased IL-38 and IL-38 mRNA expression in serum and synovial membrane of RA patients compared with that in the control group

Some studies have reported that IL-15 acts synergistically with IL-17 to enhance the inflammatory response, whereas other studies have reported contrasting findings.

3.1.3 | Interleukin 17

Interleukin 17 (IL-17) is a T-lymphocyte-induced, inflammatory response-promoting cytokine with immune response, inflammatory, and hematopoietic functions. Thl7 cells and their cytokines exhibit high levels in the synovial fluid of patients with active RA and low levels after treatment. IL-17 directly mediates monocyte migration by activating monocyte chemotactic phosphorylation of the MAPK p38 pathway.[13] As a central factor in producing the Thl7 inflammatory effects and in mediating autoimmune diseases, IL-22 exerts a synergistic effect on IL-17, whereas ROR-γt is a specific transcription factor for Thl7-cell differentiation. TGF-β and IL-6 are the differentiation initiators of Thl7 cells, whereas IL-23 is vital for its maintenance. IL-1, IL-15, and IL-21 are also vital for the positive regulation of Thl7 differentiation and development, whereas IL-12, IL-27, IFN-γ, IL-4, T-bet, and SCOS3 inhibit their expressions. IL-17 upregulates its own gene expression, stimulates the secretion of inflammatory cytokines such as MMPs (MMP-3, MMP-9) and TNF-α, and amplifies the inflammatory response.[14] Additionally, IL-23 positively regulates IL-17 and plays an indirect role. In phase II/III studies, anti-IL-17 antibodies (secukinumab and ixekizumab) exhibited an improvement in the clinical symptoms of patients with RA.

3.1.4 | Interleukin 23

Interleukin 23 (IL-23), a member of the IL-12 family of cytokines, consists of a specific subunit IL-23p19 and a subunit of IL-12, p40, both of which are linked through disulfide bonds. It is mainly produced by antigen-presenting cells, such as dendritic cells,

phagocytes, and microglia, and plays a vital role in anti-infection, antitumor, and autoimmune diseases. Specific IL-23 knockout in the CIA model mice exerts a significant protective effect, suggesting that IL-23 may have a key role in joint autoimmune diseases.[15] IL-23 is involved in RA pathogenesis.[16] Active IL-23p19 is linked to IL-12p40 through disulfide bonds and send out signals through the IL-23R and IL-12Rp1 complexes. IL-23R is associated with Janus Kinase 2 (JAK2) composition, whereas IL-12Rβ1 is associated with tyrosine kinase 2 (Tyk2). IL-23R binds to STAT3 in a ligand-dependent manner, leading to its phosphorylation and activation. Activated STAT3 homodimerizes ectopically into the nucleus to induce expression of the transcription factor RORyt, which activates transcription of the downstream factors IL-17A, IL-17F, and IL-22. IL-23 expression is positively correlated with the DSA28 score, blood sedimentation, ultrasensitive CRP level, and swollen joint count (SJC). Thus, IL-23 can be used as an indicator for monitoring the efficacy of RA drugs, and it might be a novel target for RA treatment.[17] Despite the evidence of a definite benefit of IL-23 monoclonal antibodies (ustekinumab or guselkumab), patients with active RA treated with ustekinumab or guselkumab did not exhibit considerable improvements in their signs and symptoms.

3.1.5 | Interleukin 38

Interleukin-38 (IL-38) is the tenth member of the IL-1 family. It is located on chromosome 2, 2q13-14.1, which has a length of 7.8 kb and comprises five exons and 19 amino acids. Its molecular weight is approximately 18, whereas its molecular formula is $C_{757}H_{1164}N_{198}O_{226}S_9$. IL-38 is widely expressed in immune tissues of multiple organs such as basal epidermal cells and B cells in the spleen and tonsils.[18] IL-38 exhibits a high amino acid sequence homology with IL-1Ra and IL-36Ra and is presumed to act as an IL-1 receptor antagonist, with biological effects similar to those of IL-36Ra (Figure 1). IL-38 is highly associated with autoimmune diseases such as psoriasis, ankylosing spondylitis, and RA. IL-38 significantly downregulates the IL-6, IL-8, and TNF-α expressions in RAW264.7 cells and effectively inhibits LPS-induced IL-6, IL-8, and TNF-α expressions.[19] IL-38 affects RA progression by inhibiting the pro-inflammatory cytokine expression. Additionally, IL-38 is involved in regulating the IL-17 and IL-22 production by affecting Th17, which in turn affects disease development.[20] The underlying mechanism of IL-38 regulation remains poorly understood. In mouse models, IL-38 levels are increased in both serum and joint fluid. IL-38 has not yet been reported for monitoring the efficacy of RA in China. The use of IL-38 as a primary indicator of the efficacy of RA in combination with SAA and NLRP3 has been explored for its high sensitivity and specificity.

3.2 | Proteins

3.2.1 | Serum amyloid A

Serum amyloid A (SAA) is the precursor of tissue amyloid A, which is produced by the liver. SAA combines with high-density lipoprotein in plasma to exert its biological effect on cell adhesion, migration, proliferation, and aggregation.[21] SAA is an acute-phase reaction protein expressed at low levels under normal conditions and elevated levels in response to tissue injury or inflammation, rising from baseline levels up to 1000-fold in 4–6 h. Human SAA comprises 104 amino acids, with four Sa-encoding genes located on chromosome 11p15.6, which contains four isoforms, namely SAA1, SAA2, SAA3, and SAA4. The SAA1.3 allele is not only a risk factor for AA amylase-like degeneration but also a key factor for Japanese patients with RA

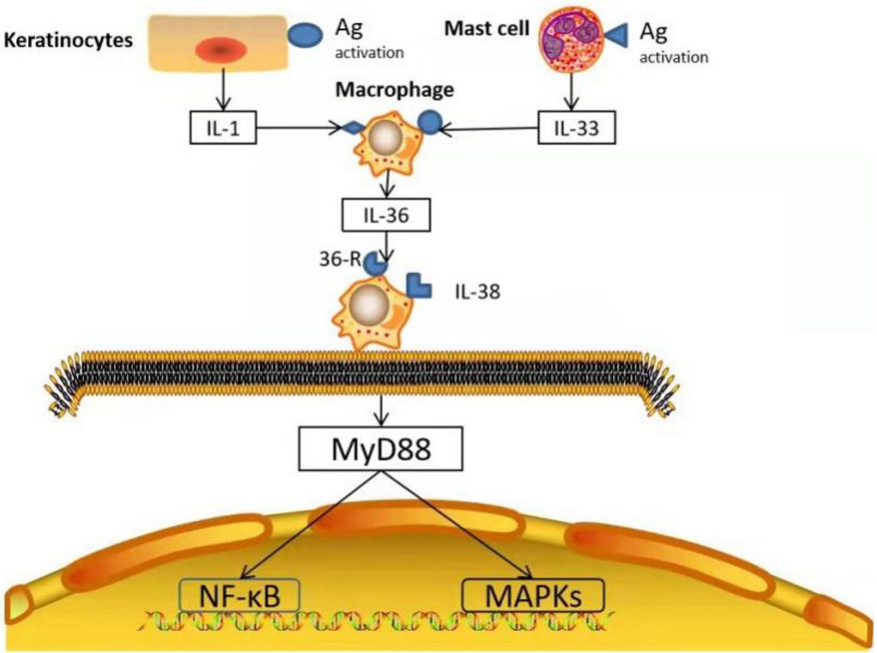

FIGURE 1 IL-38 is involved in RA pathogenesis[42]

with slow disease progression and shortened survival time.[22] SAA is highly expressed in numerous autoimmune diseases such as systemic lupus erythematosus and arthritis. SAA expression levels are increased in the serum of patients with RA and positively correlated with disease activity, CRP level, and blood sedimentation. However, the mechanisms of changes in SAA levels remain unknown. RA-induced persistent inflammatory response is associated with a sustained increase in the levels of inflammatory cytokines (IL-1), TNF-α, and IL-6, which increase SAA synthesis. Abnormal SAA processing by mononuclear phagocytes is believed to initiate the formation of AA and amyloidogenic fibrils in the lysosomes (Figure 2). Studies have exhibited that SAA is superior to CRP in terms of disease extent[23] and can replace CRP as a novel target for RA. Matrix metalloproteinases (MMPs) contribute to the hydrolytic reorganization of SAA proteins, thereby inducing neointima formation. A few studies are available on the correlation between MMPs and SAA.

3.2.2 | Matrix metalloproteinase-3

MMPs belong to the zinc peptidase superfamily, a group of Zn^{2+}-containing protein hydrolases that degrade all protein components of the extracellular matrix (ECM). The activation process is considered to be the rate-limiting factor for ECM degradation. High MMP expression is observed in the synovial membrane, synovial fluid, and peripheral blood of patients with RA and correlates with disease progression.[24] MMP9 is highly expressed in the osteoclasts of RA bone.[25] MMP3, an important member of the MMP family, is highly expressed in the sera of patients with ankylosing spondylitis, osteoarthritis, gastric ulcer, angina pectoris, knee effusion, and gastric ulcer. It can be used as an indicator for monitoring the efficacy of the condition. In RA pathogenesis, synovial cells synthesize and secrete MMP3 in the form of enzymes through stimulation of cytokines such as IL-1 and TNF-α, which are converted into active MMP3 by the action of fibrinolytic enzymes and histone G. This process activates collagen plasminogen secreted by synovial cells, which in turn activates other MMPs and triggers a "waterfall effect." The mRNA level of MMP3 increases at the joint between articular cartilage and pannus of RA, and its activation can lead to degradation of major cartilage components such as chondronectin, fibronectin, and collagen types IV, VII, IX, and XI.[26] MMP3 levels correlate with disease severity and have prognostic significance as RA markers.[26] Chinese medicine has been well researched and intensively used in RA treatment; however, none of the studies have investigated RA treatment with Western medicine. Experiments have exhibited that the serum MMP3 levels in patients with RA are higher than those in healthy groups. MMP3 is highly sensitive and representative as a monitoring indicator of methotrexate efficacy.

3.2.3 | Receptor activator of NF-κB ligand

Receptor activator of NF-κB ligand (RANKL) is a type II transmembrane protein that lacks a signal peptide and belongs to the TNF superfamily. Human-derived RANKL comprises 317 amino acids, which can be divided into two forms, namely membrane-bound and soluble, and is mainly derived from the synovial tissue and activated T lymphocytes (a few B10 cells with a $CD19^{+}CD24^{hi}CD27^{+}$ phenotype have also been reported). Activation of the TNF/TRAF signaling pathway activates the MAPK cascade and downstream JNK pathway. TRAF directly and indirectly activates IKK, thereby activating NF-κB. The secreted protein is enriched in ECM and induced by the Wnt signaling pathway. T cells produce various cytokines (such as IL-1, IL-4, and IL-6), induce RANKL expression, trigger osteoclast generation, and promote bone formation.[27] Soluble RANKL is detected through liquid-phase suspension microarray, whereas RANKL is detected on peripheral blood T cells through flow cytometry.

OPG and RANKL are involved in the bone destruction mechanism. OPG, RANK, and RANKL play a central role in bone injury, with RANK binding to RANKL to transmit signals and induce differentiation of precursor cells and osteoclasts. OPG competitively binds with RANKL to inhibit RANK activation. Feuerherm et al. detected a marked increase in OPG levels in synovial fluid and serum of patients with RA, the magnitude of which was lower than that of RANKL that increased the RANKL/OPG ratio and binding of RANK and RANKL, thereby promoting osteoclast activation.[28] RANKL concentration in synovial tissue reflects the extent of bone destruction in the affected joint. RANKL is cleaved by osteoblast-expressed MMP-3 and osteoclast-expressed MMP-7 at the bone–tumor interface. Patients with RA have increased RANKL expression and decreased production of secretory proteins (such as dickkopf-1 and DKK1). Increased RANKL expression promotes osteoclast differentiation, whereas decreased production of secretory proteins promotes bone formation. In animal studies, RANKL was reported to induce CXCL10 expression in osteoclast precursors, which in turn upregulated RANKL expression in $CD4^{+}$ T cells.[29] Schett et al[30] investigated factors such as serum RANKL levels and lifestyle habits in 115 patients with traumatic fractures and 31 patients with non-traumatic fractures. The authors observed that the proportion of B10 cells expressing RANKL in the synovial fluid of RA patients was positively correlated with the number of joints with compression pains, DAS28, and bone destruction severity, with no correlation with age, disease duration, ESR, and CRP; additionally, TNF-α was observed to promote high RANKL expression by B10 cells. Barricotini and tofacitinib inhibit JAK and TOR signaling pathways, offering new directions for RA-targeted therapy.

3.3 | Antibodies

3.3.1 | Glucose-6-phosphoisomerase

Glucose-6-phosphoisomerase (GPI) is an in vitro-secreted multifunctional protein molecule that is distributed widely in human tissues. It is involved in glycolysis and gluconeogenesis and acts as a target antigen binding to anti-GPI antibodies, forming complexes deposited on synovial surfaces and vascular endothelial cell walls, which induces arthritis through the Fc-mediated or activated complement replacement pathways.[31] Elevated concentrations of soluble GPI in serum

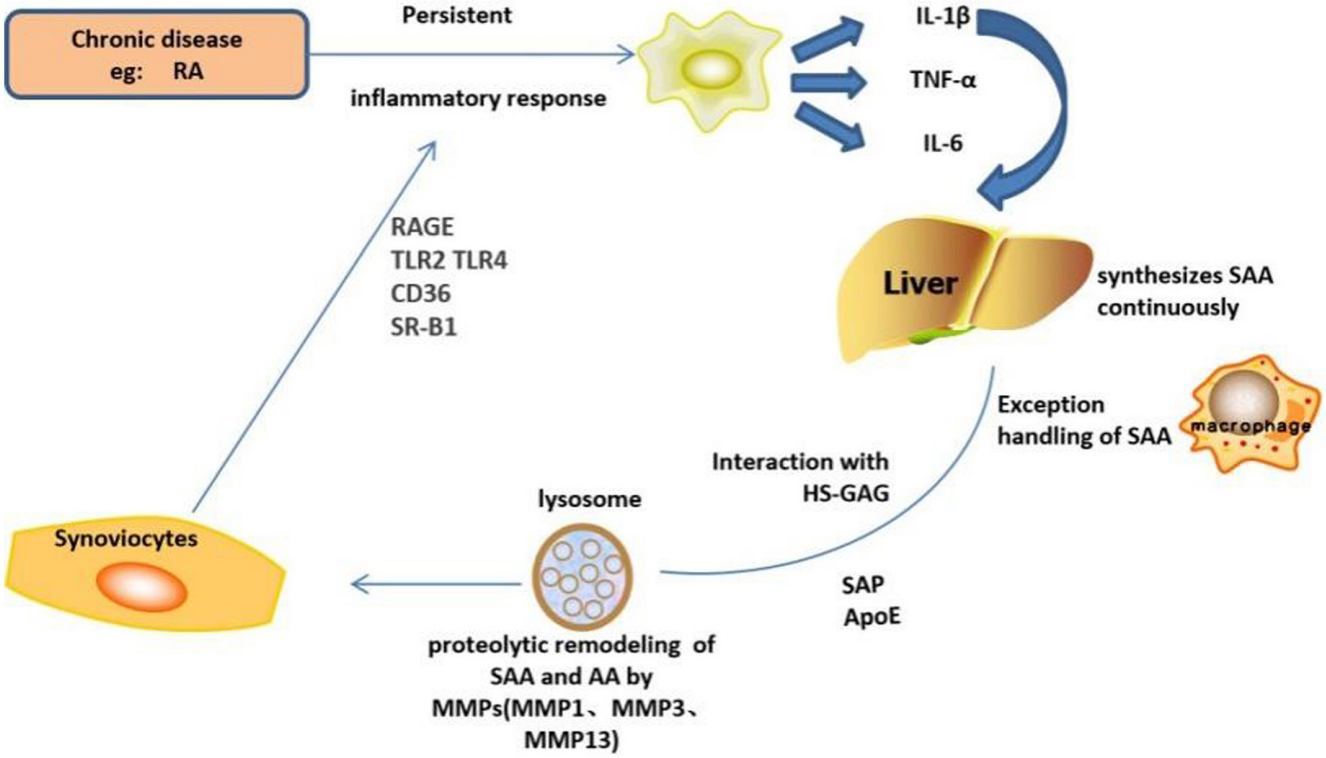

FIGURE 2 Various immune cells participate in RA pathogenesis[43]

TABLE 3 Non-cytokine indices

Classification		Source	Mechanism of action	Clinical application
Protein	SAA	Liver	SAA, TLR2, MAPKs, And NF-kB	Not found yet.
	MMP3	Synovial cells, fibroblasts, chondrocytes, tumor cells, and T lymphocytes	JNK, ROS, C-Jun, AP-1, and MMP3	TNF-α blockers (such as Infectious Etanercept)
	RANKL	Synovial tissue and activated T lymphocyte	RANK, TRAF6, MAPK, JNK, C-JUN, and NFATc1	Baricitinib Tofacitinib
Antibody	GPI	Endothelial cells	GPI, GP, Fas, and caspase-3	Nonspecific index
	AKA	Epithelial cells	Not found yet	Not found yet
Steroids	VitD3	VDR produced from B cells, T cells, macrophages, and mast cells	Vit. D3, VDR, VDRE, CAMP, and ROS	Total glucosides of paeony

and fibroblast-like synoviocytes were demonstrated in 76.1% of patients with RA. Additionally, serum GPI concentrations were higher in patients with active RA than in the inactive group patients.[32] It plays a role in RA through stimulation of fibroblast-like synovial cell proliferation, inhibiting the Fas-mediated caspase apoptotic pathway and mediating the local migration of pro-inflammatory factors into the joint. Hypoxia-induced angiogenesis is dependent on G6PI expression in human dermal microvascular endothelial cells and the secretion of VEGF in RASFs, which is also regulated by G6PI.[33] GPI titers correlate with disease activity, function grading, SJC, tender joint count (TJC), grip strength, and duration of morning stiffness, whereas it does not correlate with SHS, ERO, or JSN score.[34] Anti-GPI correlates with anti-CCP antibodies and anti-MCV antibodies. Yu et al[35] observed that GPI is involved in MMP-3 regulation in tumor cells. Schaller et al[36] detected high levels of anti-GPI antibodies (64%) with high specificity (95%) and poor prognosis in the serum joint fluid of patients with early RA. The sensitivity of GPI combined with anti-CCP antibodies is higher than that of GPI alone, whereas the specificity of GPI is slightly reduced after its combination with anti-CCP antibodies. The combination can improve the sensitivity and specificity of the diagnosis. Although the recommended indicators for early RA diagnosis are GPI, anti-MCV, and RF IgG, the finding must be validated in a large, multicenter clinical cohort.

3.3.2 | Anti-keratin antibody

Anti-keratin antibody (AKA) is an IgG antibody observed in the sera of patients with RA that reacts with the keratin layer of the upper middle segment of the murine esophagus. Its target antigen is a

TABLE 4 Non-cytokine-based indicators

Classification	Interactions between inflammatory factors	Effects on whole body and synovial/bone level
SAA	Positive correlation with disease activity, CRP, and blood sedimentation. RhSAA induces expression of the FLS pro-inflammatory factor MMP3. RANKL induces SAA.3 secretion from preosteoblastic cells in a COX-2-dependent manner. AKA response strength is positively correlated with SAA	Increased expression levels in the serum of patients with RA
MMP3	Positive correlation with ESR, CRP, joint function score, and X-ray grading severity. Inhibition of MMP3 activity, upregulation of the OPG/RANKL ratio, and control of RA disease. Positive correlation with GPI levels.	MMP3 is highly expressed in the synovial membrane, synovial fluid, and peripheral blood of patients with RA
RANKL	Positive correlation with joint pressure pain index, DAS28, and degree of bone destruction. 1,25(OH)2D3 acts directly on RANKL and inhibits its production.	Significant increase in RANKL in synovial fluid and serum in patients with RA
GPI	Correlation with disease mobility, functional class, number of swollen joints, pressure pain index, grip strength, and duration of morning stiffness. anti-GPI was correlated with anti-CCP and anti-MCV.	High levels of anti-GPI antibodies detected in serum and joint fluid of RA patients
AKA	Correlation with AI, GS, ESR, CRP, SAA, ssDNA. Correlation with disease severity. Negative correlation with VitD3.	Anti-AKA antibodies can be used as an indicator for early diagnosis of RA
25-$(OH)_2D_3$	Negative correlations with ESR, CRP, FIB, DAS28, TJC, SJC, and body mass index. Positive correlation with anti-CCP antibodies.	Significantly lower levels in serum of patients with RA

filoprotein, which is specifically secreted by epithelial cells (it is also thought to be CCP) and can be present in early RA or even before the onset of RA, thereby exhibiting greater clinical significance for diagnosis.[37] AKA assay has a sensitivity of 20%–80% and a specificity of 79%–100%, which are between those of anti-CCP and IgM-RF assays,[38] and its combination with anti-CCP and GPI assays can be used for the specific diagnosis of RA. AKA shares epitopes with HLA-DRBI and is strongly correlated with AI, GS, ESR, CRP, SAA concentration, and ssDNA, with no correlation with MS, PS, and Hb. Anti-CCP and high AKA concentrations correlate with disease severity and a poor prognosis. The assay used is indirect immunofluorescence (IIF), which is subjected to human bias and empirical influence and thus presents difficulty in quality control and standardization. Thus, it is not feasible for routine items and can be used as a differential diagnosis.

3.4 | Steroid

3.4.1 | 1, 25-Dihydroxyvitamin D3

Vitamin D (VD) is a steroid hormone and the main factor that inhibits the differentiation and maturation of thymic stromal dendritic cells (DCs).[39] The active substance is 25-hydroxyvitamin D3, which binds to the VD Receptor (VDR) to regulate immune cell proliferation and differentiation.

VD could upregulate Treg cells and downregulate Th17 cells, inhibit the synthesis of inflammatory cytokines (e.g., IL-2, IL-17, IL-23, and IFN-γ), promote the secretion of anti-inflammatory factors (IL-4, IL-10), inhibit B-cell proliferation, and reduce immunoglobulin secretion. VD interacts with the immune system in T-cell proliferation, blocking the pathway of pro-inflammatory factors (IL-6, IL-17, and IL-23) through miR-124. Active VD inhibits T-cell activation and proliferation, whereas 25-hydroxyvitamin D3 plays a potential role in RA. However, the role of reduced VD in RA onset and disease development remains controversial.[40]

In Malaysian patients with RA, 25-hydroxyvitamin D3 was significantly and positively correlated with race and body mass index (BMI) and negatively correlated with the erythrocyte sedimentation rate (ESR), CRP, FIB, DAS28, TJC, SJC, and BMI.[41] VD deficiency was observed in 63% of patients with RA and 76% of healthy controls.[41] Early VD supplementation considerably delays CIA; reduces the incidence of arthritis, severity of bone erosion, and pathology scores; decreases IgG levels; and improves bone erosion. Further prospective, multicenter studies with a large sample size are warranted to strengthen the findings of the present study.

Indicators of other non-cytogenic subcategories are shown in Table 3 and Table 4. Table 3 shows the inflammatory factors produced

by different types of cells and the regulation on them by different treatment methods. Table 4 illustrates the interactions between inflammatory factors and their effects on whole body and synovial/bone levels.

4 | PROBLEMS AND PROSPECTS

The immune system could recognize the cells and molecules of the antigen and maintain a balance between immune activation and immune suppression to protect the body from invasion. Once the balance is disturbed, immunosuppression or self-tolerance is lost, and an invasion on self-tissues initiates, leading to the development of RA. Genetics and environment are usually considered to be the main pathogenesis factors for RA. Inflammatory factors are also being investigated. One or more of the inflammatory factors mentioned in the preceding section of this article may be promising for an accurate diagnosis of RA, prediction of RA progression, efficacy monitoring, and prediction of individual response to drugs. RA is treated mainly with DMARDs such as methotrexate and leflunomide, supplemented by non-steroidal anti-inflammatory drugs. However, the complete cure has not been achieved yet. Novel therapeutic approaches such as biological or cellular therapies are constantly being explored and developed. RA treatment with cytokines and their antagonists specifically intervenes and corrects abnormalities in the immune response of RA, thus interrupting the pathological process of RA while minimizing the toxic side effects. Most studies on inflammatory factors have focused on the quantity or ratio. However, the immunosuppression mechanisms between cells are highly complex and worth investigating. The results of in vitro and animal studies are not necessarily valid in vivo, and validation of rational therapies through clinical trials is essential. Single molecules and individual proteins exert limited therapeutic effects on a single target. The 2018 China Rheumatoid Arthritis Treatment Guidelines recommend the administration of a traditional synthetic DMARDs in combination with a targeted synthetic DMARDs when treatment with a single traditional DMARDs does not yield the expected results. More studies are needed to validate the combined action of multiple inflammatory factors (e.g., MMP3 and SAA) for the diagnosis and efficacy monitoring of RA and the development of targeted drugs. Simultaneously, the complex role of NLRP3 in the development of RA and ankylosing spondylitis should be further explored, which can provide a new theoretical basis for subsequent research on drug regulation and targeting design and treatment. Future studies should focus on individualized and systemic treatments of RA, and the combination of these treatments with TCM and gene precision therapy might become a novel path in the future.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

PATIENT CONSENT STATEMENT

Not applicable.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Not applicable.

CLINICAL TRIAL REGISTRATION

Not applicable.

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