Review Article

Cytokines and miRNAs in the Pathogenesis of Rheumatoid Arthritis: A Review

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Abstract

Rheumatoid Arthritis (RA) is a chronic autoimmune disease characterized by inflammatory synovial hyperplasia. The pathogenesis of RA may be related to heredity, infection and sex hormones. The initial stage of RA involves the activation of T cells. Immature CD4+ T cells differentiate into T helper (Th) cells and T regulatory (Treg) cells under antigen stimulation and cytokine signal transduction. Cytokines secreted by Th cells and Treg cells play crucial roles in the pathophysiology of RA. The cytokines can be roughly divided into pro-inflammatory cytokines, anti-inflammatory cytokines, and both pro- and anti-inflammatory cytokines. The imbalance between pro-inflammatory cytokines and anti-inflammatory cytokines would lead to a variety of autoimmune diseases. The disease severity was significantly indicated by serum or plasma cytokine levels with RA patients. Many clinical trials have shown that anti-cytokine drugs are effective in treating RA. This article reviews the differentiation process of different Th cells and Treg cells, the roles of cytokines secreted by them in the pathogenesis of RA and how miRNAs mediate immune regulation in RA. By understanding the roles of cytokines and miRNAs in the pathogenesis of autoimmunity, it is necessary to develop potential anti-cytokine drugs and biomarkers/therapeutic targeted drugs through various ways in the treatment of RA.

Keywords: Rheumatoid arthritis; T helper cells; T regulatory cells; Pro-inflammatory cytokines; Anti-inflammatory cytokines; miRNA

Abbreviations

RA: Rheumatoid Arthritis; Th cells: T helper cells; Treg cells: T regulatory cells; Foxp3: Forkhead Box Protein 3; IFN: Interferon; IL: Interleukin; T cells: T lymphocytes; APC: Antigen-Presenting Cells; MHC-II: Major Histocompatibility Complex-II; STAT: Signal Transducer and Activator of Transcription; IL-12R: IL-12 Receptor; CXCR: CXC Motif, Receptor; CCR: C-C Chemokine Receptor; IP10: IFN-γ-Inducible 10-kDa Protein; Mig: Monokine Induced by IFN-γ; I-TAC: IFN-Inducible T cell Alpha Chemoattractant; Smads: Smad Family Proteins; TGF-β: Transforming Growth Factor-β; RORγt: Retinoid-Related Orphan Receptor γt; IRF4: Interferon Regulatory Factor 4; nTregs: natural Tregs; iTregs: induced Tregs; TIGIT: T cell Ig and ITIM domain; CIA: Collagen-Induced Arthritis; G: Guanine; A: Adenine; MSCs: Mesenchymal Stem Cells; IL-6Rα: IL6-specific Receptor α; sIL-6R: Soluble IL-6R; CSIF: Cytokine Synthesis Inhibitory Factor; vIL-10: Viral IL-10; EBI3: Epstein-Barr virus-Induced Gene 3; EAE: Experimental Autoimmune Encephalomyelitis; PIAS3: Protein Inhibitor of STAT3; VEGF: Vascular Endothelial Growth Factor
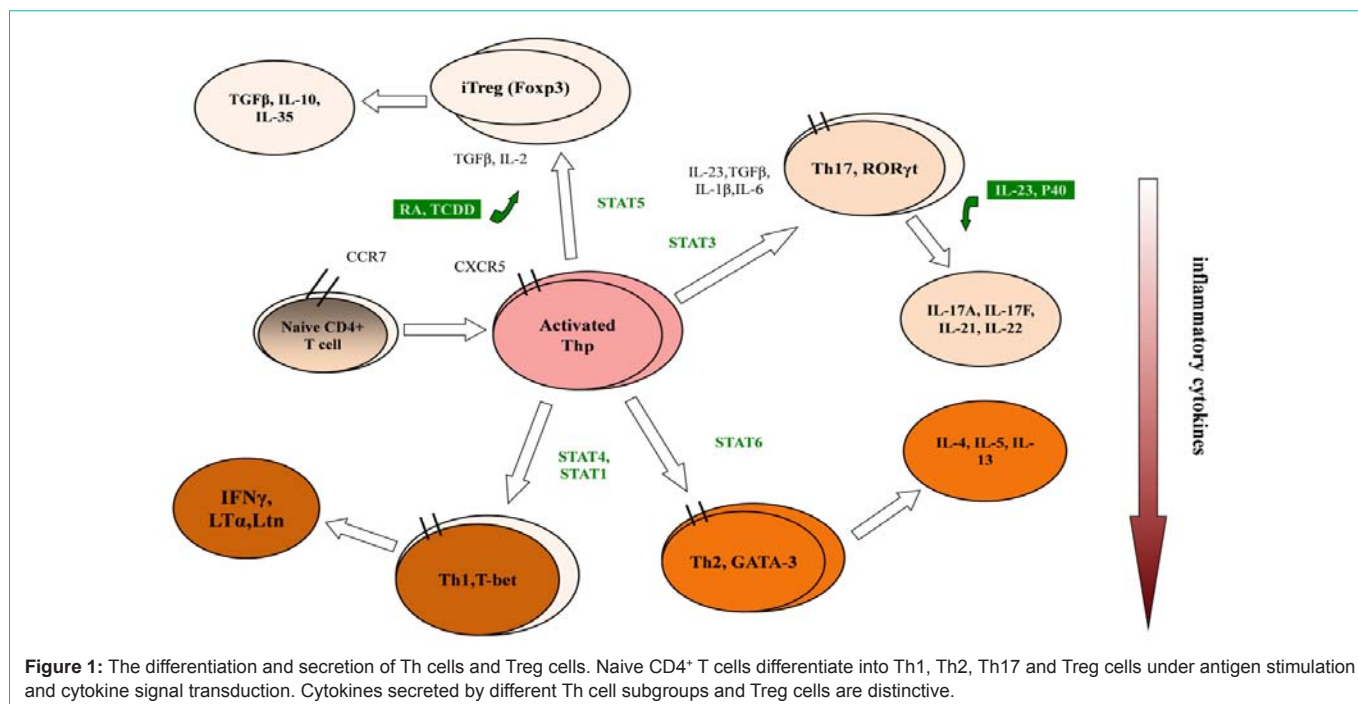
Introduction

Rheumatoid Arthritis (RA) is a chronic autoimmune disorder characterized by nonspecific inflammation of synovial membranes and joints. Accompanied by extraarticular organ involvement and serum rheumatoid factor positive, RA would lead to joint deformity and loss of function. Increasing with considerable morbidity and mortality worldwide, RA is affecting 0.5-1.0% of the general population [1]. A heavy economic pressure was brought and a social burden on the whole world by RA.

As a critical role in host defense, CD4+ T cells make major contributions to the generations of autoimmune and inflammatory diseases. Naive CD4+ T cells were differentiated into various forms of T helper (Th) cells and T regulatory (Treg) cells under such various conditions as antigenic stimulation and cytokine signaling. Since the discovery of the Th1/Th2 dichotomy by Robert Coffman and Timothy Mossman in 1986, many Th subsets were discovered. Each Th subset was found with a unique cytokine profile, functional properties and distinctively presumed roles in the autoimmune tissue pathology. Treg cells play an important role in regulating the immune response. Treg cells were lately discovered in regulating Forkhead box protein 3+ (Foxp3+) as key molecules. Another significant member of the CD4+ T cell subsets is Th17 cell; it plays a critical role in autoimmune diseases. In recent years, with the help of modern technology, the knowledge of Th17 cells was with tremendous development. Th17 cells also contribute to the pathogenesis of arthritis by modulating antibody function [2]. Manipulation of dysregulated miRNAs *in vivo* through miRNA delivery or inhibition offered a promise for new therapeutic strategies in treating rheumatic diseases [3].

CD4+ T cells & Th cells

In 1986, Robert Coffman and Timothy Mossman had a revolutionary discussion over two types of Th cells, Th1 and Th2 cells, based on the cytokine secreted by distinct CD4+ T cell subsets [4], Interferon-γ (IFN-γ) and interleukin-4 (IL-4), respectively [5]. In recent years, the classical Th1/Th2 models were hugely modified and

Figure 1: The differentiation and secretion of Th cells and Treg cells. Naive CD4+ T cells differentiate into Th1, Th2, Th17 and Treg cells under antigen stimulation and cytokine signal transduction. Cytokines secreted by different Th cell subgroups and Treg cells are distinctive.

the mechanisms of Th cell differentiation were deeply uncovered.

Originated from lymphoid cells of marrow, T lymphocytes (T cells) mature in thymus under complicated and rigorous regulations. Exiting from thymus, matured T cells carry all the genetic components to recognize antigens and express CD4 or CD8 molecules. Exogenous antigens are ingested by Antigen-Presenting Cells (APC) through phagocytosis or pinocytosis to form phagosomes. Phagosome was fused with lysosomes to form phagolysosomes. Antigens are degraded into small molecular polypeptides by proteolytic enzymes in acidic environment of phagolysosomes, among which antigen peptides are immunogenic. After entering the Golgi apparatus, the Major Histocompatibility Complex-II (MHC-II) molecules synthesized in the endoplasmic reticulum are carried by secretory vesicles and fuse with phagolysosomes to form an antigen-peptide-MHC-II complex. The complex expressed on the surface of APC can be identified and bound by corresponding CD4+ T cells. Through a complex process of blast cell transformation, the activated T cells can undergo further differentiation to various subtypes of T cells and can express different combinations of immune response genes [6].

Th1 cells

Th1 cell differentiation is generally induced by IL-12 secreted from APCs. However, IFN-γ mRNA, and only modestly augments antigen-induced IFN-γ mRNA cannot be induced by IL-12 itself from Th1 cells [7]. IFN-γ and IL-12 cytokines activate signal transducer and activator of transcription 1 (STAT1) and STAT4 respectively to promote the expression of T-bet, IFN-γ and IL-12 receptor (IL-12R) β2 [8-10]. T-bet, discovered as a novel protein belonging to the T box family by Szabo et al. in 2000 [11], is a STAT1-induced regulator of IL-12R expression in naive CD4+ T cells [9]. T-bet was served as a master regulator for Th1 cell differentiation [11]. The expression of T-bet resulted in Th1 cell development and the specific expression of IFN-γ [11] as well as IL-12Rβ2. The production of IFN-γ and IFN-

γ-STAT1-T-bet pathway construct a positive feedback loop. IFN-γ-STAT1-T-bet pathway was considered as the main mechanism of Th1 cell differentiation. It is worth mentioning that Lighvani et al. firstly found that the IFN-γ-STAT1-T-bet pathway serves as a powerful amplification mechanism in Th1 cell differentiation *in vitro* [12]. However, the IL-12-STAT4-T-bet pathway is critical both *in vitro* and *in vivo* to Th1 cell differentiation [13]. The differentiation and secretion processes of Th cells and Treg cells were illustrated in Figure 1.

In addition to IL-12 and IFN-γ mentioned above, IL-2 and TNFα are two well-known cytokines secreted by Th1 cells. Another two specific kinds of cytokine receptors expressed by Th1 cells are CXCR3 (CXC motif, receptor 3) and CCR5 (C-C chemokine receptor 5) [14,15]. CXCR3 is the receptor for the CXC chemokines IFN-γ-inducible 10-kDa protein (IP10), Monokine induced by IFN-γ (Mig) and IFN-inducible T cell Alpha Chemoattractant (I-TAC). CCR5 was first cloned from a human genomic DNA library based on its similarity to a murine C-C chemokine receptor clone in 1996. CXCR3 is present in most peripheral blood memory T cells and is expressed at higher levels in Th1 cells than in Th2 cells [16,17].

Th2 cells

The differentiation of Th2 cells abides by a similar process to Th1 cell development. IL-4 was first identified as an essential cytokine for Th2 cell differentiation. IL-4 and IL-2 cytokines activate STAT6 and STAT5 respectively to promote the expression of transcription factor GATA-3 and cMaf [18,19]. The expression of cMaf can enhance the production of IL-4, IL2Rα and IL4Rα. GATA3 serves as the first master regulator for Th2 cell differentiation [20]. In a word, STAT6 and STAT5 were activated by IL-4 and IL-2 cytokines respectively. The expression of IL-4 and IL-4-STAT6-GATA3 pathway also constructs a positive feedback loop. The feedback loop was considered as the main mechanism of Th2 cell differentiation.

In contrast to the secretion of CXCR3 and CCR5 by Th1 cells, Th2 cells were found to possess a specific chemokine receptor CCR4. CCR4 is a small, basic, structure-related molecule that regulates cell transport of various types of leukocytes by interacting with 7-transmembrane G protein-coupled receptors [21]. CCR4 was found to be strongly expressed in human T cell lines and peripheral blood T cells, whereas not in B cells, natural killer cells, monocytes nor granulocytes [22].

Th17 cells

In 2005, Laurie Harrington firstly found that Th17 cells were distinct from the T helper types 1 and 2 lineages [23]. The differentiation mechanism of Th17 cells went through a tortuous process. Initially, as a crucial cytokine, IL-23 triggers the STAT3 pathway to promote the secretion of IL-17A via its distinct IL-23R [23-25]. Subsequently, Th17 cells were highly generated when with Smad family Proteins (Smads) and STAT3, activated by transforming growth factor-β (TGF-β) and IL-6 respectively [26-28]. Then, the expression of transcription factor retinoid-related orphan receptor γt (RORγt) was induced to initialize Th17 cell differentiation [29].

However, until 2010, Ghoreschi et al. showed that Th17 cell differentiation could proceed in the absence of TGF-β signaling [30]. Another important cytokine involved in the differentiation of Th17 cells is IL-1β. IL-1β signaling promoted Th17 cell function by induction to transcription factor RORγt and Interferon Regulatory Factor 4 (IRF4) [31]. A recent study found that IL-21 could substitute for IL-6 to generate Th17 cells *in vitro* [32]. Th17 cells then produce effector cytokines IL-17A, IL-17F, IL-21, IL-22 and TNFα, among which IL-17A and IL-17F are mainly produced. Effector T cells in patients with RA are hyporesponsive due to chronic exposure to TNFα [33]. IL-21 further promotes the process of Th17 cell differentiation in a positive feedback manner, which may be similar to the roles of IFN-γ and IL-4 in the development of Th1 and Th2 cells, respectively.

Treg cells

CD4+ Tregs are composed of two types: natural Tregs (nTregs), which originate from the thymus and induced Tregs (iTregs), which are generated in the periphery. The nTregs play a role through contacts between non-cytokine dependent cells, but the iTregs work by employing the expression of inhibitory cytokines such as IL10, IL-35 and TGF-β. Differentiation environment, antigen specificities and inhibition mechanism determine the direction of differentiation of Treg cells.

In 1995, the CD25 molecule (the IL-2 receptor α-chains) was identified as the first surface marker for Tregs by Sakaguchi and his colleagues [34]. Subsequently, as a forkhead transcription factor encoded by the X chromosome, Foxp3 was indicated as a sign of Treg cells, critical in both thymic Treg cell differentiation and peripheral Treg cell differentiation [35,36]. More recently, CD127 was regarded as a candidate for Treg cells. In 2007, it was found that STAT5 activated by IL-2 is required for the development of Foxp3+ Treg cells, STAT5 binds directly to the transcription factor Foxp3 to induce the Treg cell differentiation [37]. However, it was found that the differentiation of iTregs is induced not only by stimulation with IL-2 but also by activation of TGF-β [38]. Immature CD4 single-positive thymocytes receive TCR signals of varied strength via interactions with peptide-MHC on antigen-presenting cells [38]. Beyond TCR, such co-stimulatory factors as CD28, CD80/86 (B7), CD40 and IL2Rβ are also essential for nTreg cell differentiation [39-41].

As mentioned above, iTreg cells are generated in the periphery. However, TCR repertoires displayed that most of the peripheral Treg cells originate in the thymus [42]. In 2007, it was indicated that both thymic and peripheral Foxp3- non-Treg cells expressed TCRs distinct from Foxp3+ Treg cells through experiments conducting in BDC2.5 TCR transgenic mice [43]. Results indicated that most Treg cells in the periphery are limited to particular environments or tissues. An imbalance between Foxp3 Treg cells and Th17 cells is often linked with autoimmune diseases, including arthritis [2].

Foxp3 is not sufficient for the acquisition of a stable Treg phenotype since human naive T-cells can readily express Foxp3 upon TCR stimulation [44]. The sustained expression of high levels of Foxp3 in iTreg cells is influenced by a particular mode of TCR signaling and through synergistic effect with such signals as TGF-β and IL-2. TGF-βR signaling appears to be required for most induction to Foxp3 in peripheral CD4+ T cells [45]. IL-2 is also required for TGF-β-mediated induction to Foxp3 in peripheral T cells *in vitro* [46]. IL-2 not only activates STAT5 for the development of Foxp3+ Treg cells but also opposes CD4+ T cells differentiated into Th17 cells [47].
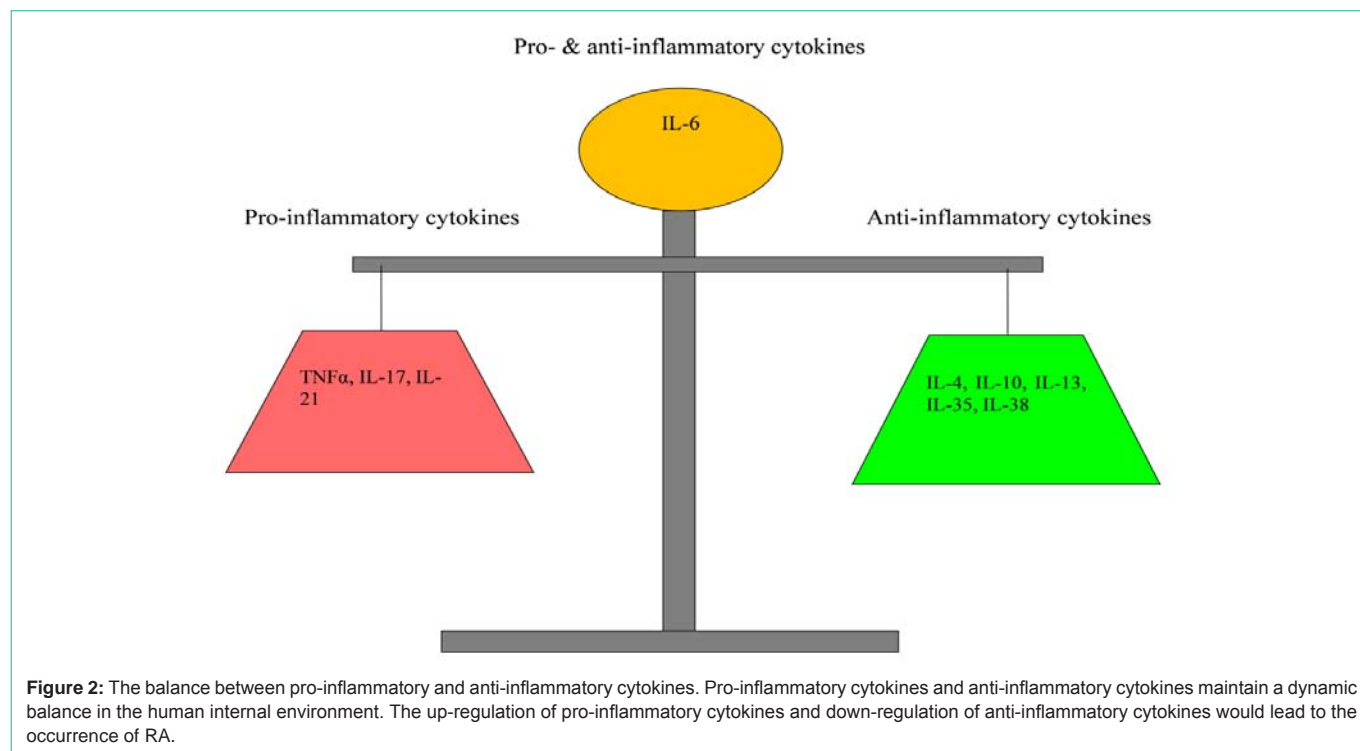
Another important factor that has been demonstrated to exert direct effects on iTreg cell differentiation is the retinoic acid. It has been documented that the all-trans retinoic acid can assist TGF-β in converting naive T-cells into Foxp3+ cells with potent suppressive activity by CD3/CD28 stimulation [48].

Different types and strength of TCR stimulation determine that CD4+CD25+ Treg cells express whether high levels of bound or soluble TGF-β. Several cell-surface molecules were proposed to play a role as mediators of Treg cell-mediated suppression, such as CD25, CD39, CD73, CD152 (CTLA-4) and a recent discovery of a novel Ig family member, T cell Ig and ITIM domain (TIGIT) [49-52]. When Treg cells interact with dendritic cells, TIGIT seems to induce dendritic cells to produce immunosuppressive cytokines IL-10 and TGF-β [53]. It was found that although retinoic acid augments Foxp3 induction inhibited the expression of IL-10. The fact indicated that one potential existing distinct differentiation cues for iTreg cells and IL-10-producing Treg cells [54]. However, it is yet unclear what the relative contributions of iTreg cells are to the mature peripheral Treg cell population, further studies will be important for dissecting the functional niches of these cells. Admittedly, the imbalance between Foxp3+ Treg and Th17 cells is often linked with RA [2].

It has now been shown that several of the drugs employed in the medical therapy of RA can partially restore Treg cell function, which has also been associated with amelioration of the clinical symptoms of RA [55]. CRISPR-mediated Treg genome editing was considered as a potential application of personalized therapy of RA [56].

Roles of Cytokines in RA

Secreted by Th cells and Treg cells, cytokines play crucial roles in such biological processes as cell growth, proliferation, differentiation, tissue repair, inflammation and regulation of the immune response [57]. The cytokines secreted by Th1, Th2, Th17 and Treg cells can be

Figure 2: The balance between pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory cytokines and anti-inflammatory cytokines maintain a dynamic balance in the human internal environment. The up-regulation of pro-inflammatory cytokines and down-regulation of anti-inflammatory cytokines would lead to the occurrence of RA.

classified three types: pro-inflammatory cytokines, anti-inflammatory cytokines and both pro- and anti-inflammatory cytokines. TNFα, IL-17 and IL-21 are pro-inflammatory cytokines. IL-4, IL-10, IL-13 and IL-35 are anti-inflammatory cytokines. IL-6 is a kind of both pro- and anti-inflammatory cytokines. IL-38 was reported to exert an anti-inflammatory function. The balance between pro-inflammatory cytokines and anti-inflammatory cytokines was illustrated in Figure 2.

TNFα

TNFα is an important pro-inflammatory cytokine with diverse functions in the immune system [58]. It was confirmed that anti-TNFα had therapeutical effects on joint pathology in both Collagen-Induced Arthritis (CIA) models of DBA/1 mice and RA patients [59,60]. Although TNFα was insufficient for the initiation step of Th17 cell differentiation, it was found to amplify the differentiation process *in vitro* [61]. On one hand, TNFα promotes the process of Th17 cell differentiation induced by IL-6 and TGF-β; on the other hand, TNFα indirectly promotes the development of Th17 by inducing IL-6 [61]. Interestingly, the IL-17 cytokine is a strong inducer of TNFα, as if they together form a positive feedback loop. In the last decade, many scholars studied the relationship between TNFα and RA from the perspective of molecular biochemistry. The transition of Guanine (G) to Adenine (A) at position 308 is the first identified polymorphism. Several studies have reported the association between the -308 TNF SNP and the susceptibility to RA [62]. The enhancements of TNFα production and thus the disease susceptibility were also associated with gene polymorphisms at positions −857 C/T, −863 C/A, −1031 T/C and +1304 G/A [63].

IL-4

IL-4 is an essential cytokine for Th2 cell differentiation, expression and secretion. It activates STAT6 to promote the expression of transcription factor GATA-3, the full development of Th2 cells and then the specific expression of IL4. In experimental animal models, it was found that IL-4 inhibits the production of inflammatory cytokines IL-1 and IL-6 in joints, thereby inhibiting the production of arthritis [64]. In a synovial fluid of some RA patients, serving as an anti-inflammatory cytokine, IL-4 hinders the formation of pro-inflammatory cytokines such as rheumatoid factor TNFα, IL-1β and IL-6 [65]. "Apoptosis resistance" was believed as the leading cause of aberrant cell survival in RA [55]. Studies *in vitro* showed that IL-4 reduces bone resorption by directly affecting osteoclasts and inhibiting the production of MMPs [65]. As the cross-regulation between Th17 and Th2 cells, IL-4 can inhibit Th17 cell differentiation and IL-17 induction [23]. In contrast, when IL-4 was blocked and independent of Th1 or Th2-related transcription factors (T-bet, STAT-1, STAT-4 and STAT-6), production capacity of Th17 cells increase *in vitro* [66]. One meta-analysis summarized that promoter polymorphism (590, T/C) of IL-4 was a useful marker for early diagnosis and treatment of RA [67].

It was reported that the potential role of vitamin D were provided a fresh perspective on RA pathogenesis and treatment [68]. To alter the frequency and function of memory lymphocytes while promoting Treg cell generation, Mesenchymal Stem Cells (MSCs) have been proposed as a candidate of choices for RA cell therapy [69].

IL-6

IL-6 cytokine is produced in many immune and stromal cells and has both pro- and anti-inflammatory properties in the immune system [70]. In the classic signaling pathway, IL-6R has two chains, namely IL6-specific receptor α (IL-6Rα) and a signal transducer (gp130). IL-6 binds to transmembrane IL-6Rα, which then associates with gp130

to activate STAT1 and STAT3 for downstream biological activities [71]. However, in IL-6 trans-signaling, soluble IL-6R (sIL-6R) lacking transmembrane and cytoplasmic regions binds to gp130 subunits. Evidence suggests that classic signaling is anti-inflammatory whereas trans-signaling is pro-inflammatory [72]. The pleiotropic cytokine IL-6 is crucial to the differentiation of Th17 cells, and the balance between pathogenic Th17 and protective Treg and that targeting the IL-6R by humanized antibodies provided new insights to improve RA [73].

IL-10

IL-10, known as human Cytokine Synthesis Inhibitory Factor (CSIF), is a kind of anti-inflammatory cytokine. It is produced by such innate and adaptive immunity cells as dendritic cells, macrophages, mast cells, natural killer cells, eosinophils, neutrophils, B cells, CD8+ T cells, Th1 cells, Th2 cells, Th17 cells and Treg cells [74]. However, IL-10 is mainly secreted by Th2 and Treg cells. It was found that Th17 cells driven by TGF-β and IL-6, were shown to express IL-10 [75]. On the one hand, IL-10 reduces such pro-inflammatory cytokines as TNFα, IL-1α, IL-1β, IL-6, IL-8, IL-12 and GM-CSF [65]. Dendritic cells promote the secretion of IL-4 and reduce the production of IL-12, resulting in Th1/Th2 imbalance in an IL-10-dependent manner [76]. IL-10 inhibits Th17 by reducing STAT3 phosphorylation and RORγt [77].

Mouse DBA1 CIA models showed that the adenovirus-mediated gene transfer and the expression of viral IL-10 (vIL-10) significantly reduced the severity of arthritic symptoms; while the pretreatment of Ab anti-IL-10 can abolish the effect of vIL-10 gene transfer on the development of CIA [78].

IL-10 deregulation plays a role in the development of such inflammatory diseases as neuropathic pain, Parkinson's disease, Alzheimer's disease, osteoarthritis, rheumatoid arthritis, psoriasis, systemic lupus erythematosus, type 1 diabetes, inflammatory bowel disease and allergy [74].

IL-13

IL-13 is a kind of anti-inflammatory cytokine mainly produced by Th2 cells. IL-13 shares a common cellular receptor (IL-4 type 1 receptor) with IL-4 [79]. IL-13 decreases the production of pro-inflammatory cytokine TNFα and IL-6 (both pro- and anti-inflammatory cytokine). As a cytokine secreted by Th2 cells like IL-4 and IL-10, IL-13 has been found similar effects on down regulating the production of IL-1β [65]. The ability of IL-13 to down-regulate cytokine secretion is equivalent to that of IL-4, but not as good as that of IL-10 [65]. However, adding IL-10 to the cultures treated with IL-4 or IL-13 reduces the production of IL-1β [65].

The IL-13 cytokine expresses higher than IL-4 in SF indicated that IL-13 plays a more important role in the regulation of synovial inflammatory responses in patients of RA [80]. Two groups of experiments conducted on CIA mice suggested that IL-13 inhibits bone resorption by suppressing cyclooxygenase-2-dependent prostaglandin synthesis in osteoblasts and subcutaneous inoculation of IL-13-secreting vector cells can significantly alleviate collagen-induced arthritis [81,82].

IL-17

IL-17 is a kind of pro-inflammatory cytokine mainly secreted by

Th17 cells. IL-17 family contains six cytokines: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E and IL-17F. Characteristic cytokines of Th17 cells, IL-17RA and IL-17RC are the corresponding receptors of IL-17A and IL-17F. After binding, the heterodimeric receptor complex initiates intracellular signaling pathways. As IL-17F shares about 50% homology in amino acid sequence with IL-17A [83], they are similarly associated with autoimmune disorders. The differentiation of Th17 cells is controlled by some cytokines (IFN-γ, IL-1β, IL-4, IL-6, IL-21 and TGF-β) and transcription factors (STAT1, STAT3, STAT4, STAT6, T-bet, GATA-3, RORγt and RORα) [84].

The IL-17 level of RA patients is higher than that of healthy volunteers and osteoarthritis patients [85,86]. IL-17 increases the production of some pro-inflammatory cytokines such as TNFα, IL1β and IL-6 [87]. A group of experiments conducted in DBA-1 mice indicated that the inflammation of synovial membrane and the damages to joint aggravated when IL-17 was over-expressed in the keen joint of collagen type II immunized mice [85,88]. However, the joint inflammation, cartilage destruction and bone erosion are ameliorated when IL-17A is neutralized by anti-murine IL-17 antibody [89]. Moreover, IL17A-deficient mice loss of the ability to activate T cells and are protected from the CIA [90]. However, compared to IL-17A, IL-17F contributes less to the arthritis pathogenesis of RA.

IL-21

IL-21 is a kind of pro-inflammatory cytokine mainly produced by Th17 cells and Th cells [32]. Belonging to the common γ-chain family, IL-21 is homologous to IL-15, IL-2 and IL-4 [91]. According to the process of Th17 differentiation described above, either IL-6 plus TGF-β or IL-21 plus TGF-β would activate some transcription factors to initialize Th17 cell differentiation. It was found that IL-21RFC chimeric protein significantly reduces the severity of the RA disease in CIA mice and AA rats [92]. However, two groups of experiments conducted in Experimental Autoimmune Encephalomyelitis (EAE) models demonstrated that the differentiation of Th17 cells was greatly affected in the absence of IL-6 rather than IL-21 [93]. It was suggested that IL-21 plays a supporting role in the process of Th17 cell differentiation, but not indispensable.

IL-35

As a heterodimer of IL-12 family, IL-35 is found as anti-inflammatory cytokine secreted by CD4+ Foxp3+ Treg cells. IL-35 consists of the IL-12α (P35) and the Epstein-Barr virus-induced gene 3 (known as EBI3) subunits [94]. P35 and EBI3, which are binding to the IL-35 receptor IL-12Rβ2 and gp130 respectively, would lead to the activation of STAT1 and STAT4 proteins [95]. It was shown that T cells that expressed IL-12Rβ2 induced only phosphorylated STAT4 in response to IL-35, whereas T cells that expressed gp130 induced only phosphorylated STAT1 [95]. The regulatory activity of Treg cells decreased *in vitro* and *in vivo* of EBI3 or P35 deficient mice, which indicated that the expression of IL-35 is necessary to achieve the optimal function of Treg cells [95].

It was demonstrated that the treatment with IL-35 could ameliorate the severity of CIA mice although further studies are required to clarify its roles in RA [96,97]. However, the mechanisms of how IL-35 alleviates arthritis are different in the two groups. In one CIA model, it was found that IL-35 inhibited the production of IL-17 and enhanced the synthesis of IFN-γ [97]. In another CIA model,

IL-35 treatment increased the number of Treg cells and IL-10, and it suppressed the development of Th1 and Th17 cells [96].

IL-35 induces the expansion of Tregs and mediates the suppression of Th17 cell differentiation. IL-35 also upregulates osteoprotegerin and suppresses RANKL, thus inhibiting bone resorption. Thus, IL-35 was thought of as a potential immunomodulator in autoimmune rheumatic diseases [98].

IL-38

As a newly discovered IL-1 family cytokine, IL-38 is expressed in several tissues and secreted by various kinds of cells. IL-38 was reported to exert an anti-inflammatory function. The IL-38 family is a composite of IL-36R, IL-1RAPL1 and IL-1R1 to block binding with other pro-inflammatory cytokines and to inhibit subsequent signaling pathways [99]. Inflammatory autoimmune diseases are characterized by an imbalance between Ths, especially Th1s, Th17s and Tregs [100]. Many abnormal expressions of IL-38 in inflammatory autoimmune diseases (rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus, psoriasis) were found to be associated with Th1s, Th17s and Tregs [101]. IL-38/IL-36R and/or IL-38/IL-1RAPL1 axis primarily play an anti-inflammatory role in the development and resolution of inflammatory autoimmune diseases, and IL-38/IL-36R and/or IL-38/IL-1RAPL1 axis was indicated a possible therapeutic benefit of IL-38 in these diseases [101].

miRNAs Mediate Immune Regulation in RA

miRNAs are a class of small noncoding RNAs that are only 21-25 nucleotide long. Many studies have shown that miRNA dysfunction is closely related to RA. The initiation of RA pathogenesis is activation of the innate immunity mediated by DCs following recognition of exogenous stimuli or autoantigens. Aberrations in miR-34a in DCs may predispose to RA development. A recent study has shown that CD1c$^+$ DCs from RA patients are hyperactivated with elevated miR-34a expression in association with a reduced level of the tyrosine kinase receptor AXL, an important inhibitory DC auto-regulator and a direct target of miR-34a [102]. Over-expression and gene silencing of miR-34a in human DCs confer respective hyper- and hypo-production of inflammatory cytokines including TNF and IL-6 upon TLR stimulation. Moreover, miR-34a deficient mice are resistant to collagen-induced arthritis with reduced DC-T cell interaction *in vivo*. Interestingly, there is a selective impairment in the induction to Th17 in miR-34a$^{-/-}$ mice, suggesting that miR-34a is critical to arthritis induction, at least in part, via DC-induced Th17 expansion. It was showed that miR-34a is an epigenetic regulator of DC function that may contribute to RA [102].

Indeed, the higher Th17 frequency and/or the higher Th17/Treg ratio have been observed in RA patients [103,104]. On the other hand, the down-regulation of miR-21 in RA patients would lead to an increase in the Th17/Treg ratio [105]. miR-21 was verified as MaR1 downstream miRNA, which was upregulated by MaR1, modulating the Treg/Th17 balance and thus ameliorating the RA progression. Thus, MaR1 was argued as a therapeutic target for RA, likely operating through effects on the imbalanced Treg/Th17 ratio found in the disease [105]. Maresin 1 improves the Treg/Th17 imbalance in RA through miR-21 [105]. The elevated Th17 frequency of RA patients was showed to correlate with an overexpression of miR-

301a-3p, whereas an inverse relationship with its potential target protein inhibitor of STAT3 (PIAS3) [106]. Dendritic cells from RA patients' peripheral blood induce Th17 cell differentiation via miR-363/Integrin αv/TGF-β axis [104].

The elevation of miR-16 was correlated with Th17/Treg cell imbalance and high Th17 cell frequency in RA patients [107]. Overexpression of miR-498 suppresses Th17 cell differentiation in RA patients [108]. miR-16 participates in the occurrence and development of RA by regulating the expression of such cytokines as TNFα, IL-8, IL-6 and IL-4, and affects the proliferation and differentiation of Th17 cells and Treg cells as well [109]. Upregulated expression of miRNA-16 correlates with Th17/Treg cell imbalance in patients with RA [107]. miR-301a-3p and Th17 cells play an important role in the process of RA [106]. Decreased expression of miRNA-21 correlates with the imbalance of Th17 cells and Treg cells in patients with RA [103].

miRNA-146a$^{-/-}$Foxp3$^+$ Treg cells mediate IFN-γ-dependent lesions in a variety of organs because of STAT1 expression [110]. These Tregs produce such inflammatory cytokines as IFN-γ, TNFα and IL-17 [111]. The diminished expression of miRNA-146a upon stimulation in Treg cells of RA patients correlates inversely with disease activity and its direct target, STAT1 expression [112]. Treg/inflammatory T cell plasticity is promoted by the inflammatory milieu in autoimmune conditions as well as miRNA-146a insufficiency [113]. miRNA-155 modulation was thought of as a potential therapy for RA [114]. miRNA-155 played an essential role in the pathogenesis of autoimmune arthritis in mice [115]. Decreased expression of miRNA-146a and miRNA-155 contributes to an abnormal Tregs phenotype in patients with RA [112]. In all, miRNA-155 is a sort of important miRNAs in RA pathogenesis.

That transfection of miRNA-155 inhibitors to B cells from RA patients increased supported the functional interference of miRNA-155 in RA. Similarly, miRNA-155 is also upregulated in RA synovial monocytes and macrophages [116]. miRNA-155 was showed highly expressed in B cells from synovial tissues of RA patients [117]. Overexpression of miRNA-155 in RA monocytes also leads to higher expression of such chemokines as CCL3, CCL4, CCL5 and CCL8 [118]. Overexpression of miRNA-155 in monocytes leads to a downregulation of the negative regulator SHIP-1 and an increase in TNFα, IL-6 and IL-1β [119]. miRNA-10a down-regulation also accelerates the NF-κB-dependent pathway involving IκB, IRAK4 and TAK1. The up-regulation of miRNA-143 and miRNA-145 in RA synoviocytes renders the cells more sensitive to TNFα and Vascular Endothelial Growth Factor (VEGF) stimulation [120].

Conclusion

Although the exact pathogenesis of RA is not yet clear, the increase of pro-inflammatory cytokines and the decrease of anti-inflammatory cytokines play an important role in the pathophysiology of RA. Great efforts have been made to study the pathogenesis of RA, but the treatment was still limited to alleviating the inflammatory reaction of joints, delaying irreversible bone destruction, protecting the functions of joints as much as possible and ultimately achieving the goal of reducing disease activity. Biological agents that have been used in the clinic include Infliximab, Etanercept, Adalimumab, Tocilizumab

and Rituximab, which make the condition of most patients with RA well controlled or even completely alleviated. Because cytokines are key factors in RA, it is necessary to develop more cytokine-targeted therapies and ensure that these drugs are both effective and safe in the treatment of patients with RA. Besides, if we use multiple cytokine-targeted therapies, this will undoubtedly be very gratifying. miRNAs act a critical role in autoimmunity, specifically on their regulatory roles in the pathogenesis of several rheumatic diseases including systemic lupus erythematosus, rheumatoid arthritis and spondyloarthritis. Thus, based on cell therapeutic approaches, miRNAs were thought of as potential biomarkers/therapeutic targets for future treating RA.

Highlights

•   CD4+ T cells can differentiate into various forms of Th cells and Treg cells.

•   Cytokines can be classified as three types according to their inflammation.

•   The imbalance between pro-inflammatory and anti-inflammatory cytokines leads to RA.

•   Many studies show that miRNA dysfunction is closely related to autoimmune diseases.

•   miRNAs can be viewed as potential biomarkers/therapeutic targets for treating RA.

Acknowledgment

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