Interleukin-34: Regulator of T Lymphocytes in Rheumatoid Arthritis

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ABSTRACT

Interleukin-34 (IL-34) is a pleiotropic cytokine, which is implicated in various autoimmune diseases. Interestingly, clinical studies have found that IL-34 is markedly upregulated in the serum and synovium of patients with rheumatoid arthritis (RA), giving rise to a growing interest in understanding the role of IL-34 in RA. Although several studies demonstrated that IL-34 levels closely correlate with the disease severity, the function of circulating and synovial IL-34 in RA is still largely elusive. IL-34 was originally identified as a ligand of the colony-stimulating factor-1 receptor (CSF-1R), which is crucial for the survival, proliferation, and differentiation of mononuclear phagocytes (e.g. monocytes, macrophages [Mφ] and dendritic cells [DC]). Of note, these IL-34-responsive cells are antigen-presenting cells, which bridge innate and adaptive immunity (e.g. through priming and instructing T lymphocytes). Thus, synovial IL-34 expression has been associated with the regulation of synovial Mφ and linked to pathologic T-cell activation in RA. In this review, we discuss the current state of knowledge on the role of IL-34 in RA and, especially, focus on the impact of IL-34 on T lymphocyte activation in RA based on findings from translational and clinical studies.

Abbreviations: CSF-1: Colony-Stimulating Factor-1; CSF-1R: CSF-1 Receptor; DC: Dendritic Cell(s); HLA: Human Leukocyte Antigen; IFN: Interferon; Mφ: Macrophage(s); LITAF: lipopolysaccharide-Induced TNF factor; LPS: lipopolysaccharide; PBMC: Peripheral Blood Mononuclear Cell(s); PTP-ζ: Receptor-Type Tyrosine-Protein Phosphatase Zeta; RA: Rheumatoid Arthritis; Teff: Effector T Lymphocyte(s); TNF-α: Tumor Necrosis Factor Alpha; Treg: Regulatory T Lymphocyte(s)

Introduction

Rheumatoid Arthritis (RA) is a common inflammatory autoimmune disease with complex etiologies, which primarily affects joints. RA is primarily caused by self-reactive immune responses and involves a variety of immune cells, including (1) innate immune cells (e.g. macrophages [Mφ] and neutrophils), which are the first responders in inflammation; and (2) adaptive immune cells (e.g. B and T lymphocytes), which trigger highly effective secondary immune responses targeting specific antigens. Mφ, neutrophils, and effector T lymphocytes (Teff) are prominent immune cells in the synovium of RA patients [1]. Interestingly, several alleles of human leukocyte antigen (HLA) class II histocompatibility antigen, DRB1 beta chain (encoded by HLA-DRB1) have been highly associated with increased incidence of RA and higher inflammatory activity in early RA [2-5], indicating that the interaction between antigen-presenting cells and T lymphocytes followed by an aberrant T-cell activation is a key mechanism in the pathogenesis of RA.

Recently, the monocyte/Mφ/dendritic cell (DC) cytokine IL-34 has been suggested as a biomarker and therapeutic target in RA [6-8], supported by clinical studies demonstrating that IL-34 is robustly elevated in the serum, synovial fluid, and tissue in patients with RA [8-15]. However, while increasing evidence indicates that IL-34 plays an important role in the pathogenesis of RA, the precise mechanism underlying the increased levels of circulating and synovial IL-34 in RA [6], as well as the exact role of IL-34 in the pathophysiology of RA remains elusive. Although several functions...
of IL-34 have been described, the immunological role of IL-34 is still under debate, as IL-34 can be both pro- and anti-inflammatory depending on the clinical setting [16-21]. Notably, several studies have proposed a role of IL-34 in the regulation of T lymphocytes in RA. In this review, we discuss the current state of knowledge of the role of IL-34 in RA and, especially, focus on the impact of IL-34 on T-cell regulation in RA.

**IL-34 In RA**

IL-34 is a ligand for the colony-stimulating factor-1 receptor (CSF-1R), which is essential for the maintenance of mononuclear phagocytes, including monocytes, Mϕ, DC in tissue homeostasis and inflammation [22,23]. Studies have identified fibroblast-like synoviocytes (FLS) as a source for IL-34. IL-34 was found to be produced by FLS upon stimulation with tumor necrosis factor-alpha (TNF-α) *in vitro* [10]. CSF-1R has another ligand, which is colony-stimulating factor-1 receptor (CSF-1; also known as M-CSF). Although IL-34 and CSF-1 bind to the same receptor and display overlapping functions [24,25], both ligands show no structural similarities [26]. CSF-1R is expressed on cells of mononuclear phagocyte lineage (e.g. monocytes, Mϕ, DC) and myeloid precursors. Besides, CSF-1R is also detected in other cells (FLS [15] and osteoclasts [27-29]), which are also implicated in RA, indicating that the role of IL-34/CSF-1R axis may not be restricted only to the immune system. Recent studies identified two more receptors of IL-34, which are receptor-type tyrosine-protein phosphatase zeta (PTP-ζ) [30] and syndecan-1 [31].

However, our knowledge of IL-34 functions mediated by these receptors is limited. So far we only know that: (1) PTP-ζ activation by IL-34 induces phosphorylation of focal adhesion kinase (FAK) and paxillin in glioblastoma cells, which in turn restrain signaling pathways associated with proliferation, clonogenicity and motility [30]; (2) IL-34-mediated syndecan-1 activation modulates CSF-1R signaling pathways, but enhancing the migratory capacity of myeloid cells [31]. Collectively, increasing evidence indicates that IL-34-mediated activation of PTP-ζ and syndecan-1 has a negative effect on CSF-1R signaling. It is possible that IL-34 directly acts on T and B lymphocytes via PTP-ζ (and syndecan-1), as PTP-ζ protein was detected in T and B lymphocytes in renal biopsies from mice with advanced lupus nephritis [32]. Interestingly, Mϕ were also positive for PTP-ζ in this study [32], although transcriptional PTP-ζ expression is not evident in vitro generated bone marrow-derived Mϕ before and after stimulation [Supplemental Figure 1 in [16]]. The immunological functions of IL-34-induced PTP-ζ and syndecan-1 are so far unclear and deserve future investigations.

Nevertheless, the effects of IL-34/CSF-1R axis on Mϕ are unambiguous as CSF-1R signaling has been well studied for its impact on Mϕ biology [33] and is known to be important for Mϕ expansion in arthritic tissue. In RA, Mϕ number positively correlates with the disease severity [34-36]. Along these lines, local IL-34 injection during collagen-induced murine arthritis was associated with RA pathology [37]. IL-34-dependent mechanisms, which may support RA pathogenesis, are suggested as follows: (1) expanding Mϕ [16, 22, 23] (2) inducing T-helper cell (Th17) response via activation of monocytes/Mϕ or FLS in vivo [10,15,37]; (3) activating inflammatory pathways in FLS [15]; and (4) promoting osteoclast formation [10]. However, as mentioned in the previous section, IL-34 has double-edged roles in immunity [16-21]. Accordingly, recent studies documented a dual role for IL-34: (1) polarizing Mϕ towards an immunosuppressive phenotype in different disease models [38]; and (2) expanding IL-34-stimulated Mϕ and potentiating regulatory T (Treg) cells suppressing Th cells [17,39].

**The Impact of IL-34 on T Lymphocytes**

The initial events in the pathogenesis of RA comprise two steps: (1) activation of the antigen-presenting cells including DC, Mϕ and activated B cells; and (2) the subsequent priming of T lymphocytes with arthritis-associated antigens [40]. Th17 cells and IL-17 are closely associated with the pathogenesis of RA [41]. Shortly, after it became evident that IL-34 expression is elevated in the blood and synovium of RA patients, Tian et al. reported that the presence of recombinant IL-34 induces the upregulation of IL-17 in peripheral blood mononuclear cells (PBMC) isolated from patients with RA [11]. This was the first study, which suggested a link between IL-34 and Th17 cells (Table 1). Similarly, the link between IL-34 and IL-17 expression is also found in other inflammatory diseases, such as Sjögren’s syndrome, where IL-34 is suggested to regulate monocytes and/or Mϕ, which are involved in the pathogenesis [18]. Over the last three years, several studies have focused on identifying the molecular mechanisms that underlie the link between IL-34 and IL-17 and found that IL-34 stimulates FLS [15,42] and/or monocytes [42,43] to express IL-17 (Table 1).
Table 1: The impact of IL-34 on T-cell regulation (chronological order).

<table>
<thead>
<tr>
<th>Affected T cell</th>
<th>IL-34-stimulated cell</th>
<th>Effects</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th17</td>
<td>Human PBMC from patients with RA</td>
<td>Upregulation of IL-17 protein (ELISA) after stimulation with recombinant IL-34</td>
<td>[11]</td>
</tr>
<tr>
<td>Th17</td>
<td>Human RA FLS</td>
<td>Upregulation of IL-6 expression in RA FLS leading to the increased numbers of Th17 cells in an RA FLS-CD4+ T co-culture system</td>
<td>[15]</td>
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<tr>
<td>Th17</td>
<td>Systemic administration (Peritoneal injection)</td>
<td>Upregulation of IL-17 expression in the synovium of mice with collagen-induced arthritis</td>
<td>[37]</td>
</tr>
<tr>
<td>Th17</td>
<td>Human monocyte cell line THP-1</td>
<td>Upregulation of IL-6 (plus IL-23, IL-21, IL-1b) expression in THP-1 leading to the increased numbers of Th17 cells in a THP-1-CD4+ T co-culture system</td>
<td>[43]</td>
</tr>
<tr>
<td>Th1+17</td>
<td>Chicken Mφ cell line HD11 and fibroblast cell line OU2</td>
<td>Upregulation of Th1 (IFN-γ) and Th2 (IL-17, IL-12p40)</td>
<td>[42]</td>
</tr>
</tbody>
</table>

Wang et al. demonstrated that (1) IL-34 acts on human FLS in an autocrine manner by activating the JNK/P38/NF-κB pathway leading to the upregulation of IL-6 and IL-34 increases the number of Th17 cells in an RA FLS-CD4+ T co-culture system [15]. Similarly, the same group showed that ex vivo CD4+ T lymphocytes co-cultured with IL-34-stimulated monocytic cell line THP-1 give rise to more Th17 T cells as compared to those co-cultured with unstimulated cells [43]. However, it is to note that IL-34 alone cannot activate pro-inflammatory signaling pathways in monocytes, as shown by numerous studies [16,24,25,33], suggesting that different pathways may be involved in FLS versus monocytes leading to the same T-cell response. In line with clinical studies, systemically administered IL-34 was found to aggravate the severity of arthritis and upregulate IL-17 expression in the collagen-induced arthritis model in mice, indicating that circulating IL-34 can instruct synovial cells to produce IL-17 [37].

However, Garcia et al. inconsistently reported that exogenous addition or blockage of IL-34 has no effect on the production of inflammatory mediators in the synovium of mice with collagen-induced arthritis [34]. Most recently, Truong et al. studied the role of IL-34 in the chicken system and showed that IL-34 is a pro-inflammatory cytokine in this system inducing a variety of inflammatory mediators including all types of interferons (IFN-α, β, γ), pro-inflammatory interleukins (IL-1β, -12p40 and -17) and lipopolysaccharide (LPS)-induced TNF factor (LITAF) in both chicken fibroblast and monocyte cell lines [42]. However, no similar study in humans or mice reported that IL-34 has such effects on monocytes; the observations in the chicken system might be system-specific. Future studies need to address how circulating IL-34 is produced and whether circulating IL-34 has other additional effects in RA (more discussion in the next section).

Novel Roles of IL-34 In T-Cell Regulation

Recent studies suggest a novel role of IL-34 in immunosuppression [38]. Bézie et al. demonstrated that IL-34-activated Mφ expand and potentiate Treg cells suppressing Teff cells in transplant recipients [17]. Treg cells, a population of CD25+CD4+ cells expressing the transcription factor FOXP3 can effectively suppress T-cell activation in RA [44]. Indeed, disturbed Treg/Th17 balance [45] or Treg deficiency [46] is prominent in patients with RA. Of note, (spleen) CD8+ regulatory T lymphocytes and human FoxP3+ T lymphocytes were found to express IL-34, suggesting a paracrine loop between Mφ and regulatory T lymphocytes [17].
ly, IL-34-expanded Treg cells display a stronger immunosuppressive activity compared to non-IL-34-expanded Treg cells [17]. As IL-34 is prominent in RA patients, there is a growing interest to target IL-34 in RA [6,7,24,47,48]. To develop a successful therapeutic approach based on IL-34, we need to more precisely understand the function of IL-34 in RA. Future studies need to address whether IL-34-expanded RA-specific Treg cells can be used in the therapy for RA.

**Conclusion**

It is clear that the severity of RA positively correlates with the number of synovial Mφ. Mφ play an important role in RA pathogenesis. The increase in the number of sublining Mφ in the synovium is an early hallmark of active RA, and high numbers of Mφ are a prominent feature of inflammatory lesions [49]. Indeed, Mφ have been implicated in driving pathology in a variety of inflammatory diseases [50,51]. Of note, the RA synovium expresses both key Mφ survival factors, IL-34 and CSF-1: While CSF-1 is expressed in the synovium sublining, IL-34 is detected in the sublining and additionally in the intimal lining layer [34]. Therefore, there is a consensus that IL-34 and CSF-1 need to be targeted simultaneously to remove pathologic Mφ in the RA synovium [34,47]. Interestingly, recent studies by Guillonneau and colleagues shed light on the novel immunoregulatory role of IL-34 [17,39]. They demonstrated that IL-34 expands Mφ and potentiate Treg cells. Indeed, Treg cells are cells of therapeutic use [52,53], and it would be interesting for the future to study whether IL-34 administration along with partial Mφ depletion provides an effective combination therapy facilitating a sustained improvement of patients living with RA.

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**Disclosures**

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**References**

Tregs (+) Expanded Human Non-Cytotoxic CD8 m 458

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