Cytokine & Growth Factor Reviews 24 (2013) 515–522

Contents lists available at ScienceDirect

Cytokine & Growth Factor Reviews

journal homepage: www.elsevier.com/locate/cytogfr

Mini review

Targeting T-helper 9 cells and interleukin-9 in autoimmune diseases

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ARTICLE INFO

Article history:
Available online 5 October 2013

Keywords:
Th9
IL-9
Autoimmune
Therapeutic

ABSTRACT

CD4(+) T helper (Th) cells are central to the modulation of immune responses, with distinct effector subsets defined by their lineage-specific transcription factor expression, cytokines production and immune function. Th9, one of the recently defined subsets of Th cells, are identified by the potent production of interleukin-9 (IL-9). Recent studies have indicated that Th9 cells and IL-9 are closely associated with several autoimmune diseases, such as systemic lupus erythematosus (SLE), experimental autoimmune encephalitis (EAE) and systemic sclerosis (SSc). In the present review, we will briefly discuss the biological features of Th9/IL-9 and summarize recent advances focusing on the role of Th9/IL-9 in the pathogenesis and possible treatment in autoimmune diseases using anti-Th9 target.

1. Introduction

T cells, especially CD4+ T cells, have been implicated in mediating many aspects of autoimmune inflammation. Following activation, CD4+ T cells are usually differentiated into different cell subsets with distinct biological activities. Development of the specialized type of each of the CD4+ T-cell subsets is controlled by specific cell–cell interactions and cytokines existed in the microenvironment, which regulates the differentiation by driving the expression of particular transcription factors. The transcription factors produced subsequently control the expression of the repertoire of surface-bound and soluble factors that dictate cell function, as well as chemokine receptors and adhesion molecules that regulate the localization to specific tissues. Thus, different CD4+ T cell subsets can be defined by lineage-specific transcription factors expression, cytokines production, and subsequent immune function [1].

It has been confirmed that several CD4+ T cell subsets and their cytokines play a pleiotropic role in the pathogenesis of autoimmune diseases. Initially, IFN-γ-producing Th1 cells and IL-4 producing Th2 cells, more recently, IL-17-producing Th17 cells, IL-22-producing Th22 cells and T follicular helper (Tfh) cells have been implicated in the pathogenesis of autoimmune diseases, such as systemic lupus erythematosus (SLE) [1–10], rheumatoid arthritis (RA) [10–16], multiple sclerosis (MS) [10,17–21], ankylosing spondylitis (AS) [16,22–24], and psoriasis [10,25–30]. Conversely, our studies indicate Foxp3+ regulatory T cells can be induced by TGF-β and IL-2 and could restrain the functional activities of Th1, Th17 and Th22 [31–34], suggesting its function of preventing autoimmune disease.

While whether Th1 or Th17 cells are the key culprit of these diseases remains controversial, recent studies have demonstrated that, a new effector T cell subset, Th9 cells, which preferentially secrete interleukin-9 (IL-9) is likely involved in the pathogenesis of several autoimmune diseases including systemic lupus erythematosus (SLE) [35,36], systemic sclerosis (SSc) [36] and experimental autoimmune encephalitis (EAE), the most commonly used experimental model for human MS [37]. The pleiotropic function and signaling pathway of Th9 cells as well as IL-9 have recently been reviewed extensively [38–42]. In this review, we briefly discuss the biological features of Th9/IL-9 and highlight the recent advances focusing on the roles of Th9/IL-9 in the pathogenesis of autoimmune diseases, as well as its clinical implications and therapeutic potential.

2. IL-9 and its receptor

IL-9, cloned more than 20 years ago, was initially thought to be a Th2-specific cytokine. It was originally identified in mice as a T

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http://dx.doi.org/10.1016/j.cytogfr.2013.09.001

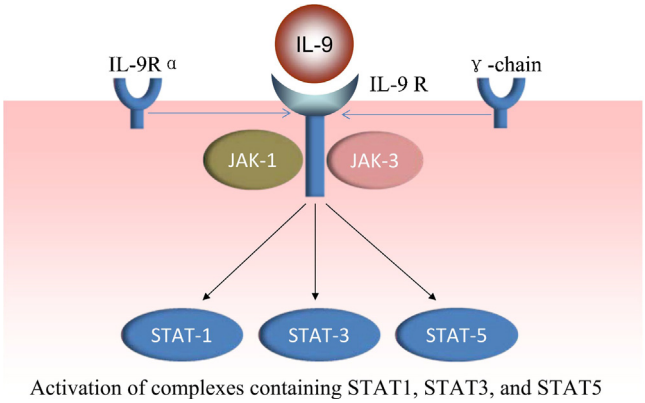

Fig. 1. The IL-9/IL-9R signaling.

cell growth factor and is a member of the common γ-chain-receptor cytokine family. The IL-9 gene loci in both humans and mice have a similar organization, consisting of five exons, and sharing a 55% amino acid homology at the protein level. The IL-9 receptor consists of the cytokine-specific IL-9 receptor α-chain (IL-9Rα) and the γ-chain [39]. IL-9 exerts its biological activity through the interaction with a heterodimer receptor complex composed of IL-9Rα and a common γ chain (γC). IL-9Rα is a specific IL-9 receptor chain produced in both soluble and member-bound forms. However, the γC chain is shared by IL-2, IL-4, IL-7, and IL-15 receptor complex [43]. Previous studies have shown that IL-9R is expressed on many cell types, such as macrophages [44], mast cells [45], dendritic cells [46], microglia [47], immature neurons [48], NK cells [49], NKT cells [50], Th17, Treg cells [51] and Th9 [52]. The wide expression of IL-9R on the multiple cell types is consistent with the diverse activities of IL-9. Although the precise signaling pathway of IL-9/IL-9R axis and its biology function remains to be unclear, signal transduction in IL-9/IL-9R has been shown to play critical roles in cell growth, survival, and differentiation of many cell types [53]. In addition, the association of JAK1 with IL-9R and the presence of IL-9-induced activation of complexes containing STAT1, STAT3, and STAT5 have been noted in several studies [39,53–55] (Fig. 1).

3. Cellular resources of IL-9

The production of interleukin-9 (IL-9) by CD4+T cells has gathered renewed interest as the result of the observation that its expression is broader than originally thought (Table 1). IL-9 is initially thought to be a cytokine made by Th2 cells which mainly express IL-4, IL-5 and IL-13 [56]. Indeed, the levels of IL-9 expression were high in Th2-prone BALB/c mouse strain and low in Th1-prone C57BL/6 mouse strain during infection with Leishmania major [57]. In addition, T cells are the main producer of IL-9 *in vivo* and that the IL-9 levels correlated with the expansion of Th2 cell population. Furthermore, the blockade of IL-4, a crucial mediator of Th2 cell differentiation, could suppress the production of IL-9 [57].

In addition to Th2, Th17 cells may also secrete IL-9 *ex vivo* [51,58]. Beriou et al. reported human Th17 cells can secrete IL-9, and long-term culture of human Th17 resulted in the marked co-expression of IL-9 and IL-17A [59]. Stephens et al. also found that human and mouse Th17 cells are a key source of IL-9. The expression of IL-9 by Th17 cells was strictly dependent on the presence of TGF-β and IL-1β, and inhibited by IL-4 [60]. Regulatory T cells (Tregs) may also produce IL-9. A study by Lu et al. linking mast cells to peripheral tolerance demonstrated that natural Tregs and inducible Tregs, both Foxp3 populations, secrete IL-9 [61]. However, there are contradictions regarding production of IL-9 from human Tregs [59,62]. Apart from Th2 cells, Th17 cells and Tregs, IL-9 was also found to be produced by NKT cells [63,64]. More recently, a study by Wilhelm et al. observed that IL-9 could be produced by innate lymphoid cells in response to papain sensitization *in vivo*, they also demonstrated that these cells are the main source of IL-9 in the lungs of allergen-sensitized mice [65].

Emerging evidence suggests that there might be a specialized new subset of CD4+ T cells dedicated to producing IL-9, in which IL-9 is regulated by a number of cytokines and transcription factors. Two concurrent publications in 2008, by the groups of Dardalhon et al. [52] and Dardalhon et al. [66], showed that IL-9 is also produced exclusively by a subpopulation of Th cells that was designated "Th9." TGF-β and IL-4 had been shown to enhance IL-9 production from activated T cells [67]. Naïve CD4+ T cells primed in the combination of TGF-β and IL-4 or Th2 cells additionally cultured in TGF-β produced high levels of IL-9 but demonstrated significantly diminished expression of other lineage-specific cytokines and transcription factors [52,66]. The results indicated that a combination of IL-4 and TGF-β, as growth and differentiation factors, promotes the development of a CD4+ T cell subset that preferentially produces IL-9, the subset was therefore named Th9 cells. Through FACS-based analyses and quantitative real time polymerase chain reaction (PCR), they determined cytokines produced by Th9 cells, and confirmed that Th9 cells they previously claimed are a discrete Th cell subset from Th1, Th2, Th17, and regulatory T (Treg) cell. Additionally, Th9 cells do not express subset-determining transcription factors like T-bet (Th1), GATA-3 (Th2), RORγT (Th17), or FoxP3 (Treg cells) at levels comparable to the respective T cell subsets, suggesting that Th9 cells are an autonomous Th cell subset [52,66]. After the identification of Th9, lineages of Th9 cells have since been gradually established by several other groups [68–70], especially by the findings of two transcription factors that are crucial for Th9 generation, *i.e.*, PU.1 [68] and interferon-regulatory factor 4 (IRF4) [69]. Deficiency in either one of the two factors impaired the generation of Th9 cells, the combinational expression of both PU.1 and IRF4 is assumed to synergistically promote IL-9 production in Th9 cells [71].

4. Regulation of Th9 differentiation and IL-9 production

Differentiation of Th9 cells can be obtained by a combination of TGF-β and IL-4, each cytokine may lead to the induction of transcription factors that regulate IL-9 production, and the expression of other genes associated with the Th9 phenotype [40]. The TGF-β signal, which induces Foxp3, also induces the expression of PU.1 [72], a key transcription factor in the Th9 development [68]. Previous studies have reported that the

Table 1
The main cellular resources of IL-9.

Cell type	Transcription factor	Stimulating cytokine	Cytokines secreted by the cell type
Th2	GATA-3	IL-4	IL-4, IL-5, IL-13, IL-10 IL-9
Th17	RORC(human) RORγt(mouse)	IL-6 + TGFβ	IL-17,IL-17F, IFN-γ, IL-21, IL-9
Treg	FOXP3	IL-2 + TGFβ	TGFβ, IL-10, IL-35, IL-9
Th9	IRF4, PU.1	IL-4 + TGFβ	IL-10, IL-4, IL-9