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Chapter

Therapeutic Potential of IL-9 in Allergic and Autoimmune Diseases

Ahmed Ummey Khalecha Bintha, Amani Souwelimatou Amadou, Mursalin Md Huzzatul and Muhammad Fauziyya

Abstract

Interleukin-9 (IL-9) is a pleiotropic cytokine produced by several immune and epithelial cells. Recently, many studies have eluded the physiological and pathological roles of IL-9 and its lineage-specific helper T cell subset (Th9). In this chapter, we will focus on the immunological role of Interleukin 9 (IL-9) in allergy and autoimmunity. We will introduce the basics of IL-9 and describe the cells involved in the secretion, signaling, and regulation of IL-9. After establishing the background, we will discuss the pathogenesis and regulation of IL-9 in allergic and autoimmune diseases. We will conclude the chapter by providing an updated therapeutics that target IL-9 and their potential uses in autoimmune and allergic diseases.

Keywords: IL-9, Th9, multiple sclerosis, Th17, IBD, uveitis, mast cells, asthma, atopic dermatitis, food allergy, diabetes, TGF-β, ILC2

1. Introduction

Interleukin-9 (IL-9) is a pleotropic cytokine that regulates diverse immunological functions (**Figure 1**). This cytokine was first identified in the late 1980s as a T cell growth factor [1]. Because of the molecular weight of IL-9, it was initially known as P40 [2]. Later studies revealed that the observed molecular weight was due to N-link glycosylation, and actual molecular weight for this discovered molecule is 14 kDa [3]. A similar factor was also identified from Th2 cells and mast cells where it was initially named as T-cell growth Factor III (TCGF III) and mast cell growth-enhancing activity (MEA), respectively [2, 4]. Further studies revealed that both TCGF III and MEA actually represent the P40 factor [4]. In later years, considering its pleotropic roles and the redundant nomenclature the P40 factor was renamed as IL-9 [5].

The locus encoding IL9 in mouse is about 11 kb in size, and located on chromosome 13 [6]. The Il9 locus is comprised of 5 exons and 4 introns [3]. The Il9 locus encode for a precursor peptide of 144 amino acids, first 18 amino acids of which is signal sequence peptide. The mature IL-9 peptide, a single-chain glycoprotein of 126 amino acids, and similar to other cytokines of IL-2 family folds into a four-alpha-helix bundles [7]. Human IL-9 locus is present on chromosome 5 in the region q31–35 [6]. Homology between mouse and human IL-9 is about 55%, and both of them contain a conserved 10 cysteine residue to form a disulfide bond that is critical for a mature IL-9 peptide. Interestingly, three conserved non-coding sequences,

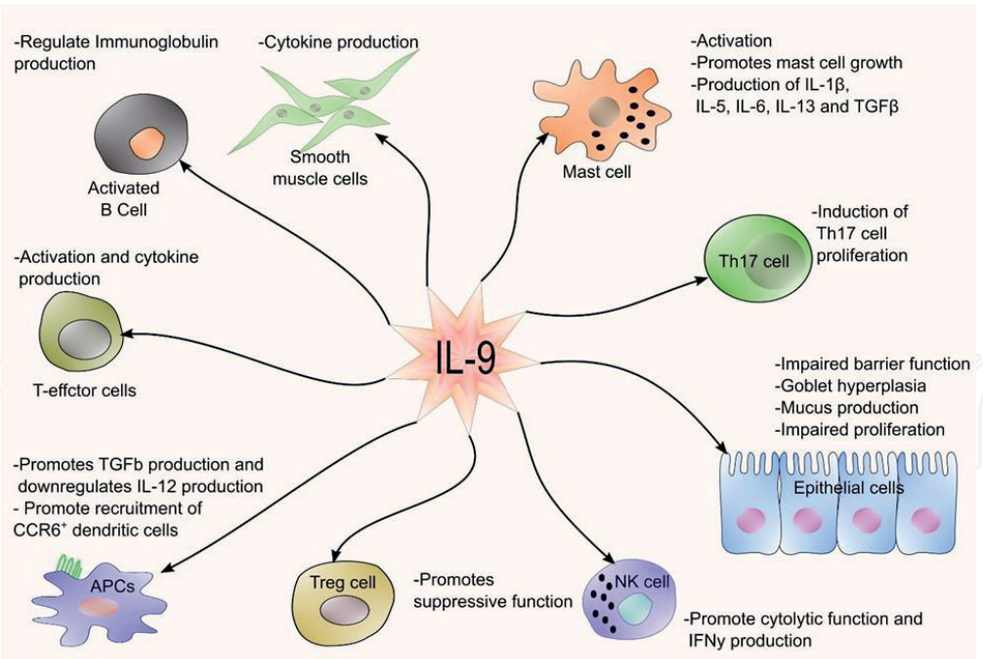

Figure 1.
Functions of IL-9. IL-9 contributes to different immunopathology and physiology through activation of multiple cell types. Illustration by MHuzzatul.

CNS0, CNS1, and CNS2 are present on both mouse and human *il9* locus sequence similarity of which is 63% [3, 7]. CNS0 is positioned in the upstream (−6 kb) of transcription start site (TSS), CNS1 is the promoter region, and CNS2 is located at the downstream of TSS (+5.4 kb) [8]. CNS1 provide binding site to numerous transcription factors that includes PU.1, STAT5, STAT6, GATA1, GATA3, IRF1, IRF4, NF-kb, BATF, AP-1, Smads 2/3/4, Gcn5, Notch [9]. Etv5 can bind to both CNS0 and CNS2, and recruit histone acetyltransferase p300 to mediate chromatin remodeling [8–11]. Regulation of IL-9 expression by this multiple numbers of transcription factors explain the necessity of a delicate cytokine milieu that requires to stimulate IL-9 producing cells. The miscellaneous origin of IL-9 and the complexity of its regulation underscore the need for a comprehensive assessment of IL-9 function. Therefore, in this chapter, we will elucidate the basis of IL-9 function in health and diseases and its therapeutic potentials in autoimmune and allergic diseases.

2. IL-9, a lineage specific Th9 cytokine

T cells were originally thought to be the main source of IL-9 [12–14]. IL-9 was defined as a Th2 cytokine. The reason for this Th2 designation by many research findings included IL-9 genome. The *il-9* gene is positioned within a Th2 cytokine clusters. Also, increased expression of IL-9 was observed in a Th2-predominate BALB/c mouse model of cutaneous leishmaniasis (BALB/c mice) but not in Th1-predominate model (using C57BL/6 mice). This finding suggested IL-9 as a Th2 signature cytokine [12]. In addition, Th2-like responses such as airway epithelial hyperplasia, proliferation of mast cells, mucin-producing cells, and eosinophils were found in the lungs of IL-9 transgenic mice [15]. More recently, the designation of IL-9 as a Th2 cytokine loses credence, due to the identification of PU.1, an ETS family transcription factor that induces IL-9 secretion. Mice with T-cell-specific deletion of PU.1 did not develop IL-9 dependent inflammation of the lungs [16]. However, the mice had similar frequencies of Th2 cells [16]. In another experiment that utilized siRNA-mediated disruption of PU.1 resulted in impaired IL-9

production in human T-cells. Recently, a distinct helper T cell subset, Th9 was identified as IL-9 lineage-specific cells. Studies observed increased PU.1 expression under Th9 polarizing conditions but not Th2 conditions [16]. The finding of another helper T cell subset suggested that Th2 is not the main source for IL-9, and PU.1 as a unique transcription factor necessary for IL-9 production emphasized the identity of Th-9. Later, *in vitro* studies identified IL-4 and TGF-β as cytokines that facilitate the differentiation of naïve T cells to Th9 cells [17, 18]. Though IL-4 is a known Th2 cytokine, TGF-β exhibit pleotropic functions and regulates the development of other helper T cells including Th17 and Treg cells [19]. Presence of IL-4 with TGF-β facilitates the differentiation of naive T cells into IL-9-secreting Th9 but not Tregs or Th17. Also, IL-4 can directly block the expression of FoxP3 in T cells thus reprogramming Treg cells into Th9 cells [17]. And, addition of TGF-β in culture medium reprograms Th2 cells to Th9 cells [18]. IL-4 and TGF-β-mediated induction of IL-9-producing cells are dependent on both activated STAT6 and GATA3, suggesting the initial identification of IL-9 as a Th2 cytokine. And Th2 including other helper T cells secrete small amounts of IL-9 [20].

3. Sources of IL-9

In addition to Th9 and Th2, other immune cells have been identified as potential sources of IL-9 (**Figure 2**). Prominent among these immune cells is Th17 cells. Th17 cells are involved in mounting immune responses against extracellular bacteria and fungi and are implicated in autoimmunity [21]. Activation of a Th17-associated transcription factor, retinoic acid receptor-related orphan receptor-γt (RORγt) with phorbol 12-myristate 13-acetate and ionomycin (PMA) leads to IL-9 secretion [22]. Tregs have also be shown to secrete IL-9 both *in vivo* and *in vitro*, however, the role is IL-9-secreting Tregs is conflicting [23, 24]. Another recently identified source of IL-9 is Vδ2 T cells in human peripheral blood. This γδ T cell subset population can be stimulated with antigens, TGF-β, and IL-15 to produce IL-9 [24]. Mast cells, natural killer T cells (NKT) have also been found to produce IL-9. Mast cells cross-linked with IgE and inflammatory mediators like histamine produce IL-9 in the presence of IL-1β and LPS [25–29]. Stimulation of NKT cells with IL-2 leads to secretion of IL-9 [30]. A large number of infiltrating IL-9 producing NKT has been found in histological section from patient with nasal NKT cell lymphomas [31]. Decreased expression of IL-9 was observed in CD1d-restricted NKT deficient mouse model of allergic inflammation suggesting NKT cell can also promote IL-9 production *in vivo* [32]. In addition, innate lymphoid cells such as ILC2s, eosinophils, neutrophils, and osteoblasts also have been found to produce IL-9 [33–35].

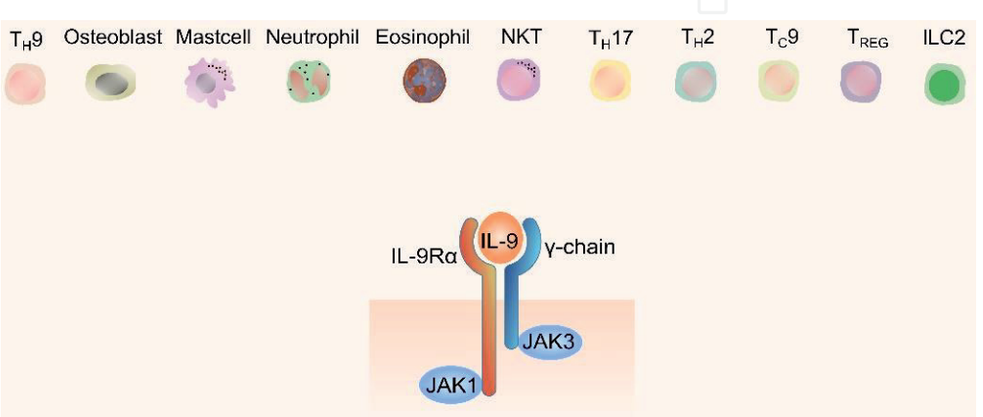

Figure 2.
Cellular sources of IL-9 and IL-9 receptor (IL-9R) heterocomplex. Illustration by MHuzzatul.

4. IL-9 receptor signaling

IL-9 exerts its biological effect on its target cells through IL-9R receptor. The IL-9R is a heterocomplex of the alpha chain (IL-9Rα) and the common gamma chain [36]. IL-9Rα is specific only to IL-9, whereas the gamma chain is present in the receptor complexes of several other cytokines such as IL-2, IL-4, IL-7, IL-13, IL-15, and IL-21 [37–39]. About 25% of the IL-9Rα exist in complex with the gamma chain outside IL-9 heterocomplex. IL-9Rα is of 522 amino acids in human, and 468 amino acids in mouse, and contains 11 exons [40]. This 64 kDa glycoprotein is a member of type I hematopoietin receptor super family due to the presence of the Box1 and Box2 motifs in the intracellular domain, and WSXWS motif in the extracellular domain [41]. Formation of a heterocomplex with the γ-chain is enhanced as IL-9 binds to IL-9Rα (**Figure 2**) [42]. The binding of IL-9 to IL-9Rα results in a conformational change in IL-9R. This conformational change recruit JAK molecules to Box1 motif which results in the phosphorylation of tyrosine residues of IL-9Rα-associated JAK1 and γ-chain associated JAK3 [41]. BOX1 motif is very critical in IL-9 mediated signaling as disruption of Box1 results in loss of

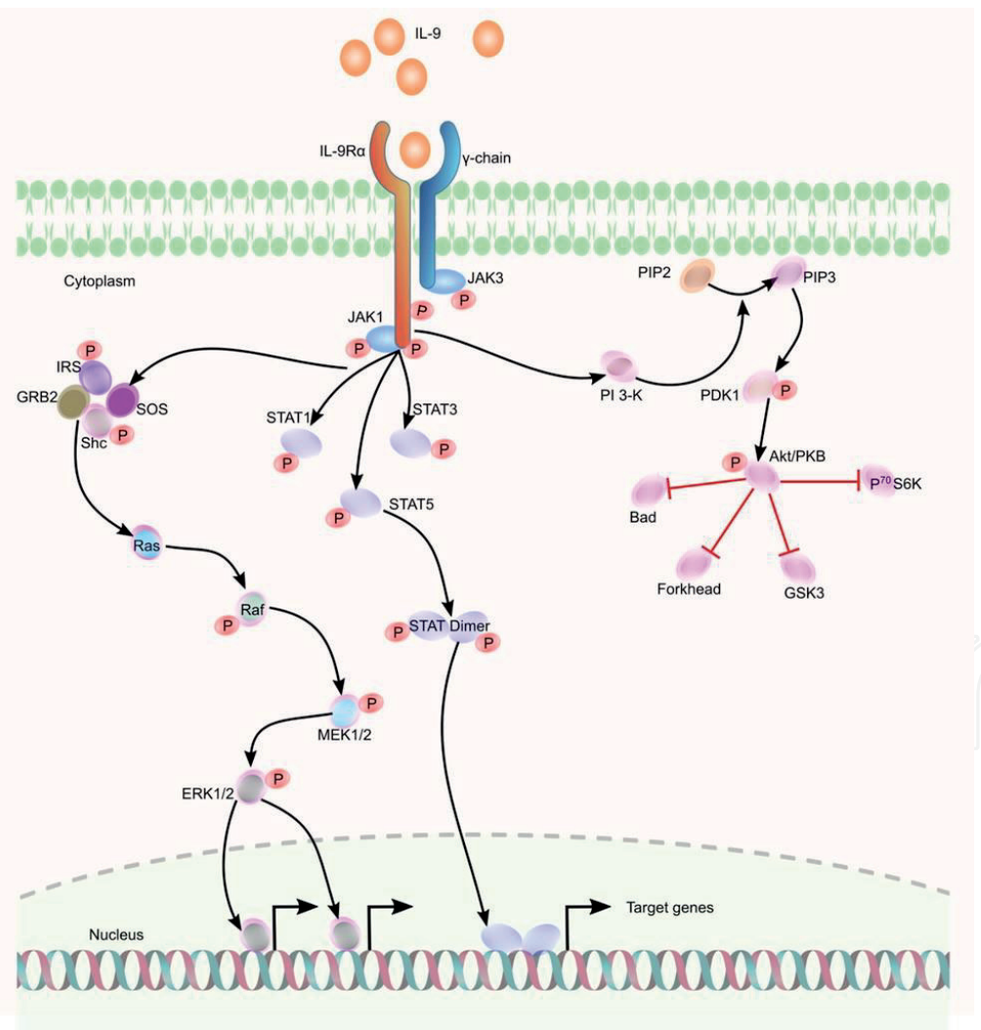

Figure 3.
Schematic representation of IL-9 signaling pathway. IL-9 cytokine binds to IL-9R complex. This leads to phosphorylation of JAKs. The phosphorylated JAKs activate STATs, PI3 kinase, and the MAP kinase pathway. IL-9R, interleukin-9 receptor; JAK, Janus kinase; STAT, signal transducer and activator of transcription; PI3K, phosphatidylinositol-3 kinase; PIP, phosphoinositide; PDK1, pyruvate dehydrogenase kinase 1; bad, GSK3, glycogen synthase kinase 3; PS6K, IRS, insulin receptor substrate; SOS, suppressors of cytokine signaling; GRB2, ERK, extracellular signal regulated kinase; Shc; Ras/Raf/MEK, mitogen-activated protein kinases; illustration by MHuzzatul.

phosphorylation of JAK1 and JAK3 [43]. Activated JAK molecules then phosphorylate a tyrosine residue (Tyr407) in the IL-9Rα, which results in the phosphorylation of intermediate molecules, STAT molecules (STAT1, STAT3, and STAT5), MAPK, and IRS-PI3 pathways (**Figure 3**) [44–46]. Activation of these pathways contribute to the upregulation of IL-9, as well as important in the growth, differentiation, and development of the IL-9 targeted cells [47, 48].

5. IL-9 and allergic diseases

Allergic diseases including respiratory, food, and skin allergies are mainly mediated by Th2 cells through the expression of various cytokines such as IL-4, IL-5, and IL-13 (reviewed in [49]). The cytokine IL-9, which was initially studied in the context of Th2-mediated immune response and later associated with T-helper 9 (Th9) cells, has been shown to play an important role in allergic inflammation [50, 51]. IL-9 and its receptor IL-9Rα regulate antibody synthesis, specifically IgE, in both murine and human B cells [52, 53]. To contribute to allergic disease pathogenesis, IL-9 also promotes activation and recruitment of inflammatory cells [54–57].

6. Asthma including airway allergies

Various studies have shown that IL-9 and its receptor contribute to airway allergic diseases and asthma. Sputum, serum, and lungs of patients with asthma were shown to have increased concentrations of the cytokine [58–60]. IL-9 levels were also increased in the airways of murine asthma models [61]. IL-9Rα is expressed on human tonsillar germinal center and memory B cells, and smooth muscles in the airways. IL-9/ IL-9Rα signaling in B cells induces STAT3 and STAT5 pathways to potentiate IgE production [52, 53, 55, 62, 63]. Overexpression of IL-9 in transgenic mice or treatment with recombinant cytokine induces expansion of B-1 cells, and accumulation of mast cells in the tissues [64, 65]. IL-9 induces the release of proteases and pro-inflammatory cytokines by the mast cells to promote survival of eosinophils and increase airway permeability [66, 67]. IL-9/IL-9Rα signaling also stimulates human airway smooth muscle to secrete eotaxin1/CCL1 and induces production of IL-13 in airway epithelial cells. Eotaxin1/CCL11 and IL-13 significantly increase eosinophil recruitment and cause lung epithelial cell hypertrophy. These effects result in asthma-like symptoms, including lung inflammation, bronchial hyper-responsiveness, and mucus accumulation. Moreover, IL-9 worsens lung injury in a murine model of chronic obstructive pulmonary disease (COPD) [63, 68, 69]. The cytokine also appears to be a critical player in allergic rhinitis. Serum IL-9 in patients strongly correlates with irritative nasal symptoms including rhinorrhea [70]. In mice, Th9 cells are significantly upregulated during allergic rhinitis and neutralization of IL-9 alleviates symptoms. Blocking IL-9 decreases the level of inflammatory cytokines (IFN-γ, IL-4, and IL-17) and eosinophils infiltration in the nasal mucosa. This causes a decrease in the frequency of sneezing and nasal rubs in experimental models of allergic rhinitis [71].

7. Food allergies

Studies in patients with food allergy and experimental oral hypersensitivity have shown that allergic reactions in the gastrointestinal tract are mediated by various players, including Th2-secreted cytokines, such as IL-4 and IL-9 [72–74]. Various

studies have shown that IL-9 drives intestinal inflammation and plays a critical role in food allergies [75, 76]. In patients with food allergies, the severity of clinical symptoms strongly correlates with increased intestinal permeability [77]. *In vitro* experiments have shown that patients with peanut allergy have increased levels of IL-9. The memory T helper cell response specific to peanuts in allergic children is dominated by IL-9. Thus, cytokine levels can be used as a biomarker to determine individuals with peanut allergy [78, 79]. In mice, overexpression of intestinal IL-9 or induction of IL-9-producing mucosal mast cells (MMC9s) also increases susceptibility to food allergy [80]. Migration of mast cell progenitors and their development into MMC9s is regulated by basic leucine zipper transcription factor ATF-like (BATF) and Th2-secreted IL-4 [81]. The large amount of MCC9s-derived IL-9 and other mast cell mediators cause intestinal mastocytosis and increased intestinal permeability, which is central to the induction of experimental oral hypersensitivity [82]. The actions of the IL-9-stimulated mast cells cause allergic diarrhea and hypothermia [75]. IL-9 can additionally be secreted by the group 2 innate lymphoid cells (ILC2) and Th9 cells to amplify the intestinal allergic inflammatory response, which may lead to anaphylaxis [83–88].

8. Skin allergies

IL-9 has been identified as a potential mediator of cutaneous allergies, including atopic dermatitis (AD) and allergic contact dermatitis (ACD). Patients with atopic dermatitis have a significantly higher level of IL-9 in the serum and skin lesions [89]. The concentration of the cytokines also positively correlates with the severity of the disease and serum IgE levels [90]. These observations were made in both adult and pediatric patients [91, 92]. A study in a Korean population also linked IL-9 and IL-9R gene polymorphisms to AD [93]. IL-9 induces IL-5 and IL-13 by ILC2. ILC2 and the cytokines are associated with AD pathogenesis. IL-5 and IL-13 contribute to the defective skin barrier in AD patients by downregulating tight junctions genes [94, 95]. IL-9 also promotes the secretion of the vascular endothelial growth factor (VEGF) by keratinocytes and mast cells [92, 96]. An increased level of VEGF contributes to the dilatation of capillaries, erythema, and inflammatory edema characteristics of AD [97, 98]. Moreover, IL-9 has been shown to regulate Th1-mediated allergic contact dermatitis. Patients with positive patch tests to nickel have a higher level of allergen-specific IL-9 expression in skin, peripheral blood mononuclear cells (PBMCs). Also, IL-9 potentially mediates infiltration of eosinophils in the skins as its levels strongly correlate with the cell infiltration in the tissues. This demonstrates a potential pathogenic role of the cytokine IL-9 in ACD [99, 100].

9. IL-9 and autoimmunity

The etiology or trigger of autoimmune diseases is not well understood [101, 102]. However, there is a consensus that many factors, including genetic, environmental, and cytokine dysregulation are implicated in causing aberrant immune responses that drive tissue damage [102–104]. Many studies on divergent immune responses in autoimmunity have shown dysfunction of helper T cell subsets, which include Th1, Th17, and/or Treg cells [104, 105]. Studies in the last decade have identified IL-9-secreting Th9 cells as another T helper cell subset involved in immune responses [23, 106]. The IL-9 cytokine has become the focus of many autoimmune studies [107, 108]. Initial studies showed IL-9

to be a growth factor and a Th2 cytokine [13, 108]. More recently, IL-9 has been characterized as a lineage-specific cytokine for Th9 cells [109]. Thereafter, many immune cells involved in autoimmunity, such as Th17 and Treg cells, have demonstrated secretion of IL-9 [16, 110]. In EAE, a rodent model of MS, researchers identified Th9 and its signature cytokine, IL-9, in driving the disease process [111]. Its close association with Th17 and TGF-β has renewed interest in the role of IL-9 in the pathogenesis of autoimmune diseases [23]. In this section, we will examine the role of IL-9 in some autoimmune diseases such as multiple sclerosis (MS), systemic lupus erythematosus (SLE), inflammatory bowel diseases (IBD), rheumatoid arthritis (RA), and uveitis.

10. IL-9 and IL-17 dynamics in autoimmunity

The role of IL-9 in autoimmunity was illuminated when many studies reported that IL-9 and IL-17 are intricately related in driving the pathogenesis of diseases [111]. Human and animal studies revealed that Th17 cells secrete some amount of IL-9, in addition to other proinflammatory cytokines [112]. During the differentiation of naive T cells, TGF-β, a key driver of Th17 polarization, plays an important role in the differentiation of Th9 cells [23]. This was well elaborated in a study by Nowak *et al* in which *in vitro* polarization of MOG-specific Th17 cells was shown to generate IL-9-secreting Th9 [22, 113]. Secretion of IL-9 was further enhanced by the addition of IL-1β or IL-21 to the culture [113]. In addition, TGF-β and IL-6 induce Th17 cells that co-express IL-9 and IL-17 [22]. Studies have shown an increased frequency of memory CD4 cells that co-express IL-9 and IL-17 in patients with Type 1 diabetes [23].

On the other hand, IL-9 potentiates Th17 functions in an autocrine manner on Th17 cells [22, 110]. Th17 is a predominant helper T-cell subset that expresses IL-9 receptors (IL-9R) [22] . Through this receptor, IL-9 acts as an activator of Th17 cells [22]. IL-9 also synergizes with TGF-β to differentiate naive T cells into Th17 cells [110]. The presence of IL-9 in T cell cultures leads to the expansion of Th17 cells [110]. The importance of IL-9 in Th17 cell function is emphasized in IL-9R-deficient experimental autoimmune encephalomyelitis (EAE) model. Mice that lack IL-9 signaling showed decreased Th17 cells and defective migration of Th17 cells into the CNS [22, 114]. Neutralization of IL-9 led to attenuation of disease in EAE [22]. This unique relationship between IL-9 and Th17 provides the premise to examine the role of IL-9 in Th17-mediated autoimmune diseases.

11. Multiple sclerosis (MS)

Most autoimmune diseases like MS occur due to alteration of immune responses, which leads to tissue damage. The importance of IL-9 in MS has been enhanced through our understanding of the roles of IL-9-secreting T cells in EAE, an animal model of MS orchestrated by helper T cells [115]. Most studies revealed IL-9 plays a pathogenic role in EAE [22]. Th9 cells and Th17 cells were observed in the central nervous system (CNS) during EAE [115]. Blockade of IL-9 signaling in EAE resulted in contradictory conclusions. One study reported increased severity of disease in IL9Ra KO mice on a C57BL/6 background through a loss of Treg function and increased secretion of GM-CSF [116]. Other studies showed attenuation of disease and decreased Th17 cell infiltration into the CNS of SJL mice treated with IL-9 blocking antibody [22, 117]. This opposing view in disease outcome may be due to differences in the helper T cell composition and dysfunction driving the

pathogenesis in the mouse strains. Also, IL-9 has been shown to increase chemokine CCL20, which enhances migration of Th17 into the CNS [22]. Accumulation and activation of mast cells during the Th17-IL9 immune response could explain the feedback loop [113]. Adoptive transfer of IL-9^{+} Th9 into recipient mice resulted in EAE [118]. Th9-EAE model manifested a unique disease profile independent of Th1 and Th17 EAE models [118].

The role of IL-9 in MS patients is complex. A study by Roucco *et al* showed that IL-9 activates STAT1 and STAT 5, which are inhibitors of Th17 function [119]. IL-9 directly interfered with IL-17 expression in Th17 cells. Levels of IL-9 in the cerebrospinal fluid (CSF) of relapsing and remitting MS patients were inversely correlated with the disease pathogenesis and the disability indices [119]. These findings suggested the immunoregulatory role of IL-9 in MS. In another study, CSF of MS patients showed increased amounts of IL-9, and levels of IL-9 correlated well with IL-17 [120]. Therefore, more studies are needed to understand the functional role of IL-9 in MS.

12. Uveitis

Unlike other autoimmune diseases, uveitis is a heterogeneous disorder that results in inflammation of the eye [121]. In animal models of uveitis, adoptive transfer of *in vitro* polarized Th9 cells induced ocular inflammation [122, 123]. However, IL-9 was not detected in the eyes or lymph nodes of these mice [123]. Analysis of inflammatory cytokines in the vitreous humor of patients with uveitis detected increased levels of IL-9, among other proinflammatory cytokines [124]. However, the biological relevance of increased IL-9 in the study was not elaborated.

Another study examined the role of IL-9 in patients with Vogt-Koyanagi-Harada (VKH) disease. VKH is a systemic autoimmunity that manifests with bilateral panuveitis [125]. Patients with active disease had significantly higher levels of IL-9 in culture supernatants and higher IL-9 mRNA in PBMCs than did healthy controls and inactive patients [126]. The synergy of IL-9 and IL-17 was demonstrated in the study. The secretion of IL-17 by IL-9-treated PBMCs of active patients was significantly higher compared to the controls or inactive patients [126]. In a study that evaluated the serum of patients with Behcet's disease, another complex autoimmune disease with uveitis, serum IL-9 was neither elevated in disease state nor correlated with disease index [127]. More studies are needed to understand whether IL-9 signaling plays any immunological role in the eye.

13. Rheumatoid arthritis (RA)

The study of IL-9 in RA highlights its functional relationship with Tregs. In an antigen-induced animal model of arthritis, mice that lacked IL-9 had a chronic disease [128]. Treatment with rIL-9 resolved the joint inflammation, swelling, and tissue damage. The absence of IL-9 led to impaired suppressive functions of Treg cells [128]. Type 2 innate lymphoid cells (IL-C2) are documented to express IL-9 and have an anti-inflammatory function [128, 129]. These studies highlight the role of IL-9 in the resolution of inflammation in arthritis [130]. In human studies, IL-9-producing IL-C2 cells were also identified in the PBMCs of RA patients [130, 131]. In a study of treatment-induced remission of RA, synovial fluid of patients showed high levels of IL-9 [128].

14. Systemic lupus erythematosus (SLE)

Proinflammatory cytokines are generally believed to be involved in the pathogenesis of SLE. High levels of IL-9 mRNA and Th17 cells were seen in SLE patients compared with healthy controls (HC) [132, 133]. Dantas *et al,* evaluated the level of IL-9 in SLE and observed that patients with SLE had elevated IL-9 compared with levels in healthy individuals [134]. Further, IL-9^{+} CD4 cells were more abundant in patients with SLE [132]. Serum IL-9 and mRNA of IL-9 were significantly elevated in SLE patients [132]. Also the elevated serum IL-9 and mRNA correlated with the SLE severity index [132, 135]. Animal studies corroborated these findings. Spleens and kidneys of lupus-prone mice showed high expression of IL-9 [136]. Neutralizing antibodies of IL-9 decreased kidney manifestation of SLE (lupus nephritis) and decreased anti-dsDNA antibody titers in these animal models [136].

15. Inflammatory bowel disease (IBD)

Aberrant adaptive immune response to the gut epithelial cells involving both CD4 and CD8 is implicated in the IBD [137]. These T cells are shown to express α4/β7 integrin, which binds to MAdcam1 on the gut epithelium [138, 139]. Gut T cells including cells that secrete IL-9 have been shown to express high levels of this integrin, and they propagate inflammation in the gut [140]. Gene expression studies have highlighted IR4 and GATA3 expression on immune cells that reside in the epithelial lining of the gut [141]. IRF4 is a transcription factor that drives the induction of Th9 immune responses in the gut [141]. Animal models of colitis confirms this finding of an abundance of the IL-9-producing T cells in the gut. These T-cells-producing IL-9 are involved in breaking the intestinal barrier [142]. In a DSS colitis model, anti-IL-9 blocking antibodies suppressed mucosal inflammation, and attenuation of disease was observed [142]. Adoptive transfer of IL-9-producing T cells into Rag2 knockout (Rag2$^{-/-}$ KO) mice also induced colitis [143]. Furthermore, IL-9 was found to directly modulate the expression of tight junction proteins, claudin and occludin in the animal model of colitis [144]. This indicates that IL-9 directly inhibited membrane integrity.

Immunological assessment of patients with inflammatory bowel disease (IBD) revealed high expression of IL-9 in the lamina propria [145]. In addition to other gut-residing T cells in IBD, CD4 cells had increased production of proinflammatory cytokines, including IL-9, which drive gut inflammation [145, 146]. Elevated levels of IL-1β and IL-9 were observed in the serum of IBD patients, and these correlated with disease prognosis [147]. Epithelial cells of UC also showed high expression of IL-9 receptor (IL-9R) [147, 148]. This receptor expression is most pronounced in patients with active disease [147]. *Ex vivo* IL-9 treatment of intestinal epithelial cells from UC patients showed increased proliferation of epithelial cells and pSTAT 5 expression [110].

Together, these findings highlight the role of IL-9 in IBD and colitis models. IL-9 could serve as a therapeutic target for IBD. Mice treated with GATA 3 DNAzyme showed it directly reduced IL-9 production and some Th2 cytokines to attenuate disease [149].

16. Type I diabetes

Studies by Vasanthakumar *et al* examined the role of IL-9 in patients with diabetes mellitus (DM) [150]. They observed that memory T cells from patients

stimulated with Th17 polarizing conditions led to IL-9 production [150]. This shows that Th17 cells from DM patients have an increased ability to secrete IL-9 [23]. The study also identified TGF-β as the critical activator of IL-9 secretion [23]. TGF-β activity links Th17 and IL-9 secretion.

IL-9 appears to play both anti- and pro-inflammatory functions in autoimmunity. The functional heterogeneity of IL-9 may result from the unique cells or the microenvironment producing it. In RA, IL-9 exhibits anti-inflammatory function [128]. Studies have elaborated the anti-inflammatory function of IL-9 as it potentiates Treg-dependent immune tolerance to allografts [151]. In the gut, it is regarded as proinflammatory [142]. Some studies have shown that the expression of the activation marker CD96 on Th9 cells may explain the immunological status of the secreted IL-9 [152]. Researchers have reported that Th9 with high expression of CD96 showed a reduced ability to cause colitis compared with Th9 with low expression of CD96, which is associated with severe intestinal inflammation [152]. More studies must be done to identify the immunological heterogeneity of IL-9.

17. IL-9 as a therapeutic target

One principle of treatment of autoimmune diseases involves inhibition of mediators of inflammation. Drugs that target proinflammatory cytokines are extensively used in the treatment of autoimmune diseases [153]. Here we explore the use of IL-9 blockade as a therapeutic target in different disease conditions.

Medimmune LLC developed a humanized anti-IL-9 monoclonal antibody, MEDI-528 [154]. This humanized anti-IL-9 monoclonal antibody was indicated for use in allergen-induced asthma in adults [154]. Results from the clinical trial of Medimmune MEDI-528 showed no increased efficacy in improving respiratory functions and control of asthma compared to placebo [155]. Preclinical studies in mice showed the efficacy of blocking IL-9 in maintaining the airway [156]. Questions remain regarding why therapy directed at IL-9 failed to produce the desired response in humans. Heterogeneity of IL-9 sources and functions could explain the differences in airway response observed in this clinic trial.

18. Other potential IL-9 treatments

IL-9R inhibitor (rhIL-9-ETA) is a chimeric toxin targeting IL9 receptor [157]. These IL-9R inhibitors have efficacy in targeting malignant cells in non-hodgkin's lymphoma (NHL) and acute myeloid leukemia (AML) expressing IL9 and IL-9R [157]. However, the efficacy of this drug has not been tested in autoimmunity. Pfizer Inc. developed a JAK/STAT pathway inhibitor, CP-690550 [158]. It specifically targets and inhibits the activation of JAK 3 [158]. This treatment effectively prevents transplant rejection [158]. This drug could be beneficial in inhibiting IL-9 signaling, which depends on the JAK/STAT pathway. JAK inhibitors have been used in the treatment of RA and psoriasis [159]. UC patients that were treated with JAK inhibitors showed decreased Th9 cells [160].

BNZ 132-1-40 peptide, an antagonist of IL-2, IL-9, and IL-15 from Bioniz Therapeutics is undergoing safety and tolerability testing in patients with moderate to severe alopecia areata, an autoimmune disease of the skin that leads to hair loss [161]. However, no results from the clinical trial were available at the time of this review. Recently, FDA approved the use of BNZ-1 for the treatment of cutaneous T cell lymphoma (CTCL) [162]. These studies suggest BNZ-1 could be used to target IL-9 in diseases [163].

Other potential drug options include RDP58, which targets IRF4, a transcription factor involved in Th9 induction [164]. Interferon gamma (IFN-γ) has the ability to inhibit Th9 polarization through IL-27-dependent mechanisms [165]. Actimmune, an IFN-γ-based therapy by Horizon Therapeutics, is FDA-approved for the treatment of chronic granulomatous disease (CGD) [166]. The efficacy of inhibiting IL-9 by this drug could be tested in IL-9-related disorders.

The immune modulatory roles of IL-9 in health and diseases are important and provides a basis for exploring IL-9 as a therapeutic target. However, the divergent roles of IL-9 in promoting and inhibiting inflammation complicate definitive drug development. Some studies have highlighted the function of IL-9 in promoting immune tolerance. Future studies to understand cell-specific IL-9 regulation and function may resolve the conundrum of therapy development targeting IL-9. More studies in disease will broaden our knowledge about IL-9 function.

19. Conclusion

Significant progress has been made in our understanding of the functions of IL9 in health and diseases. For a long time, IL-9 was considered as a T cell growth factor, however, the identification of Th9 helper T cells has expanded our understanding on the roles IL-9 play in diseases. The pathogenic functions of IL-9 in autoimmunity and allergy suggest that IL-9 signaling can be targeted for therapy development. In this chapter, we focused on the function of IL-9 in different autoimmune diseases that include MS, SLE, RA, uveitis, and allergic conditions. We also highlighted IL-9-Th17 paradigm and its complexity in autoimmune diseases. Animal models of autoimmune diseases revealed contrasting roles of IL-9 and human studies are limited. Therefore, extensive animal and human research are necessary to elucidate the divergent immunological roles of IL-9. Such studies will be required for effective drug development that targets IL-9 signaling.

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Conflict of interest

The authors declare no conflict of interests.

Author details

Ahmed Ummey Khalecha Bintha[1], Amani Souwelimatou Amadou[1], Mursalin Md Huzzatul[2] and Muhammad Fauziyya[3*]

1 Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA

2 Department of Ophthalmology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA

3 Department of Neurosurgery, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA

*Address all correspondence to: fauziyya-muhammad@ouhsc.edu

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