From bench to bedside: Therapeutic potential of interleukin-9 in the treatment of asthma (Review)

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Abstract. Initially identified as a T cell and mast cell growth factor, interleukin (IL)-9 has long been recognized as an important mediator of asthma. Recently, accumulating results from transgenic mice demonstrated that systemic or lung-specific overexpression of IL-9 caused asthma-associated symptoms. Moreover, anti-mIL-9 antibody (Ab) blocking treatment alleviated disease in animal models of asthma. In light of the large quantity of data from the murine models, MEDI-528, a humanized anti-IL-9 monoclonal Ab has been produced to assess the activity of IL-9 on human asthma. In order to ascertain whether it is a successful translation from bench to bedside, the biological features of IL-9 were evaluated and up-to-date information regarding the role of IL-9 in different experimental murine models and human asthma were summarized.

Contents

1. Introduction

Asthma is characterized by recurrent and reversible airflow obstruction associated with airway hyperresponsiveness (AHR) and airway inflammation. Asthma currently affects ~300 million people worldwide, with a large socioeconomic burden (1). Until recently, the etiology and pathogenesis of asthma remained elusive. It is clear, however, that airway inflammation induced by the release of inflammatory cytokines is critical in the chronicity and progression of the disease (1).

Interleukin (IL)-9 belongs to a member of the four-helix bundle cytokine family. Initially identified as a T cell and mast cell growth factor, IL-9 has long been recognized as an important mediator of allergic inflammation (2,3). A resurgence of interest in IL-9 has been spurred by recent work demonstrating its incremental targets and broader cellular sources (4). Indeed, in asthma, a series of experimental studies have demonstrated its diverse functions. Accumulating results from transgenic mice revealed that systemic or lung-specific overexpression of IL-9 caused an asthmatic phenotype, such as eosinophilic and lymphocyte inflammation, goblet cell hyperplasia, increased mucus production, increased immunoglobulin E (IgE) production, subepithelial collagen deposition and mast cell hyperplasia (5-7). Moreover, independent studies with anti-mIL-9 antibody (Ab) blocking treatment alleviated asthma-associated symptoms (8-11). In light of the profound effects that IL-9 has on various cells, and considering the large amount of data from the murine models, MEDI-528, a humanized anti-IL-9 monoclonal Ab has been produced to assess the activity of IL-9 on human asthma (12-14).

In the present study the biological features of IL-9 will be discussed, with a focus on the role of IL-9 in different experimental murine models and human asthma.

2. IL-9 and IL-9 receptor structure

IL-9 is a 14-kDa glycoprotein consisting of 144 amino acids, including a signal sequence of 18 amino acids (15), and belongs to the four-helical cytokine family. The human IL-9 gene is located within the T helper (Th) 2 cytokine region on chromosome 5 (5q31-35), which also encodes IL-3, IL-4, IL-5, CD14 and granulocyte-macrophage colony-stimulating factor (16,17). It has been reported that polymorphisms and linkage disequilibrium in this region have close associations with the development of asthma phenotype, including bronchial hyperresponsiveness, atopy and elevated total IgE levels (18-20). IL-9 was initially identified as a Th2 cytokine

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and numerous of the initial functions of IL-9 were examined in Th2-associated models. Later on, a specialized subset of T cells dedicated to producing IL-9 termed Th9 cells was reported (21,22). Moreover, other Th subsets, including Th17, natural regulatory T cells also appear to have the potential for IL-9 production (23). Considering there are so many potential cellular sources of IL-9, the production and effect of IL-9 *in vivo* may be under a complicated regulation.

The IL-9 receptor (IL-9R), unlike IL-9, is a heterodimer. It is composed of the α-chain (IL-9Rα) and the common γ-chain receptor shared by other cytokines including IL-2, IL-4, IL-7 and IL-15 (24,25). Consistent with its pleiotropic functions, IL-9R is expressed on various cell types, including mast cells (26), macrophages (27), dendritic cells (28) and Th cell subsets Th2 and Th17 (29). Interestingly, genetic studies revealed that IL-9R is also associated with susceptibility to asthma (30-32). In 57 Caucasian families, Holroyd *et al* (30) provided evidence of linkage between a genomic region containing the IL-9R gene and asthma or AHR. Furthermore, haplotype analyses revealed that a specific haplotype (GAGC) had a protective effect against wheezing and against the development of sensitization (31). In addition, Melén *et al* (32) demonstrated that IL-9R (rs731476), in combination with IL-4 Rα (rs1801275), exerts a major influence on susceptibility to asthma.

3. Signaling pathway

Janus kinase (JAK)/signal transducer and activator of the transcription (STAT) pathway is critical for signal transduction by IL-9/IL-9R. IL-9Rα and the common γ-chain bind to JAK1 and JAK3, respectively (33). Moreover, ligation of IL-9R activates the JAK proteins phosphorylation. This leads to the downstream activation of STAT complexes. Consequently, STAT1, STAT-3 and STAT5 form homodimers, whereas STAT1 and STAT3 form heterodimers (34). Consequently, dimerized STAT molecules translocate to the nucleus, inducing the expression of effector genes. It was recently demonstrated that IL-9 induced JAK/STAT activation is crucial in cell proliferation, survival and secretion of inflammatory mediators (35,36).

In addition, there are to some extent two more pathways involved in IL-9 signaling: The insulin receptor substrate (IRS)-phosphoinositide 3-kinase and Erk mitogen-activated protein kinase pathways (37). Although they are different from the JAK/STAT pathway, these two pathways appeared to be restricted to certain cell types (37). In several hematopoietic cell lines, IL-9 was reported to induce the phosphorylation of IRS-1 and IRS-2 (33). In addition, IL-9 was able to induce a transient phosphorylation of Erk2 in the murine Th cell line TS1, the mast cell line MC9 and in Ba/F3 and 32D cells transfected with the human IL-9 receptor (38). However, further investigation is required in order to determine the downstream effectors and the biological significance of these two signaling pathways.

4. IL-9 in asthma

It has been reported that IL-9 concentrations increased in the bronchoalveolar lavage fluid (BALF) in the murine model of asthma (11). IL-9 expression has clearly been confirmed in BALF from atopic asthmatic patients using quantitative polymerase chain reaction and ELISA together with immunocytochemistry (39). Elevated levels of IL-9 have also been detected in lungs (40,41), sputum (42) and serum (43) of asthmatic patients. Moreover, serum IL-9 levels were associated with the percentage of apoptotic eosinophils, which was recognized as a predominant feature of allergic asthma (43). Consistent with its initial identification as a mast cell growth factor, IL-9R is observed on mast and polymorphonuclear cells in the lungs of asthmatic individuals but not in healthy controls (44). These results also indicate a potential role of mast cells in asthma pathogenesis. Indeed, there is compelling evidence that an IL-9-mast cell axis is central to not only the acute symptoms of asthma but also the allergen-induced chronic inflammation (10).

5. Targeting IL-9 in murine asthma models

With these observations in mind, several investigators have studied targeting IL-9 as a therapy in murine asthma models since the late 1990s (5-11,45-49). Results from transgenic mice revealed that systemic or lung-specific overexpression of IL-9 caused an asthmatic phenotype, such as eosinophilic and lymphocyte inflammation, goblet cell hyperplasia, increased mucus production, increased IgE production, subepithelial collagen deposition and mast cell hyperplasia (Table I) (5-7). It is noteworthy that systemic IL-9 overexpressing transgenic mice only appear to exhibit the asthmatic phenotype after antigen exposure (6,7), while unchallenged transgenic mice in the present study were relatively normal (6). By contrast, the transgenic mouse model of lung selective overexpression of IL-9 displayed massive airway inflammation, mast cell accumulation, increased subepithelial deposition of collagen and hyperresponsiveness without the presence of an antigen (5). The main reason for this discrepancy may be the difference between IL-9 expression levels, the location of IL-9-producing cells or possibly a more complicated temporal and spatial regulation involved in the IL-9 overexpressed mouse model. These data, appear to indicate that IL-9 may be a promising target for the treatment of patients with asthma. However, there are contradictions in the literature regarding the role of IL-9 in the fibrotic process. A study by Arras *et al* (45) revealed that the same systemic overexpression of IL-9 protects mice against alveolar fibrosis induced by silica. Additionally, an IL-9 intraperitoneal injection into C57BL/6 mice also reduced the amplitude of silica-induced lung fibr osis. Therefore, these data appear to indicate that IL-9 can either have an anti- or pro-fibrotic role in IL-9 overexpressing murine models of asthma, depending upon the localization of fibrosis and/or the varying effector cells involved. Consistent with the data from IL-9 overexpressing mice (FVB/N-TG5), two independent *in vivo* studies confirmed that intratracheal instillation of rmIL-9 into C57BL/6 mice increased eosinophils in the BAL and significantly elevated the total serum IgE (Table II) (46,47). Moreover, intratracheal administration of IL-9 significantly increased the levels of IL-5Rα in the lung of B6 mice (46). Since several authors have linked the IL-5 and its receptor to the recruitment of eosinophils to the lung and since eosinophilic inflammation is a central feature

Table I. Targeting the IL-9 gene in murine asthma models.

Models	Antigen challenge	Results	Reference
FVB/N-TG5	/	Massive airway inflammation with eosinophils and lymphocytes, increased numbers of mast cells within the airway epithelium, epithelial cell hypertrophy associated with accumulation of mucus-like material within nonciliated cells and increased subepithelial deposition of collagen.	5
FVB/N-TG5	*Aspergillus fumigatus*	TG5 mice display significantly enhanced eosinophilic airway inflammation, elevated serum total IgE, and AHR following lung challenge with a natural antigen.	6
FVB/N-TG5	*Alternaria alternata*	There are more collagen fibers and eosinophils in the lung of Tg5 mice. The concentration of the eosinophil chemoattractant RANTES and the profibrotic mediator CTGF was higher in the BAL of challenged Tg5 mice.	7
FVB/N-TG5	Crystalline silica particles	The severity of fibrosis was significantly less important in Tg5 mice than in their wild-type counterparts.	45
IL-9$^{-/-}$ BALB/c	OVA	IL-9 knockout mice developed a similar degree of eosinophilic inflammation and AHR to their wild-type littermates. Goblet cell hyperplasia and IgE production were also unaffected. Moreover, levels of IL-4, IL-5, and IL-13 in the BAL were comparable between wild-type and knockout mice.	48
IL-9$^{-/-}$ BALB/c	OVA	IL-9 was not required for the bronchial accumulation of mast cells	49

IL, interleukin; OVA, ovalbumin; IgE, immunoglobulin E; AHR, airway hyperresponsiveness; CTGF, connective tissue growth factor; BAL, bronchoalveolar lavage.

of atopic asthma, it is conceivable that IL-9 also serves a significant role in eosinophilic inflammation in asthma.

However, in the IL-9-deficient mouse model, McMillan *et al* (48) revealed that IL-9 is not obligatory for the pathophysiological features of the allergic pulmonary response-AHR and eosinophilia. Goblet cell hyperplasia, sera total and ovalbumin (OVA)-specific IgE production and Th2 cytokines including IL-4, IL-5 and IL-13 levels in BAL were also unaffected by the lack of IL-9 (48). Data from Pae *et al* (49) also indicated that IL-9 was not required for the bronchial accumulation of mast cells. Collectively, these observations indicate that there are other alternative pathway substitutes for IL-9 in the development of asthma. Four independent studies with anti-mIL-9 Ab treatment in an OVA-sensitized mouse model revealed significantly reduced AHR, the numbers of eosinophils and lymphocytes, which resembles the classical features of asthma (Table II) (8-11). Discordance between published observations in IL-9 gene-deficient mice and an Ab blocking study may be attributable to protocol differences. As any knockout mice were generated during the embryonic stage, disruption of IL-9 at this early stage may have developed other adaptations.

6. Targeting IL-9 in asthma patients

Despite the conflicting results from murine models, the overall effects observed in these models indicated that targeting IL-9 might offer a novel approach to the treated patients with asthma. MEDI-528, a humanized anti-IL-9 monoclonal Ab has been produced to assess the activity of IL-9 on human asthma. Recently, MEDI-528 had been demonstrated with an acceptable safety profile and a linear pharmacokinetic (PK) profile when administered intravenously or subcutaneously (12-14).

In a phase IIa study, 36 mild asthma patients (18-65 years), enrolled to receive MEDI-528 (0.3, 1 and 3 mg/kg) or placebo subcutaneously twice weekly for 4 weeks, revealed that MEDI-528 had no effect on the pulmonary function (13). However, another study in 9 adults (18-50 years) with stable, mild to moderate asthma and exercise-induced bronchoconstriction (EIB) received 50 mg MEDI-528 or placebo subcutaneously twice weekly for 4 weeks, and indicated that blocking IL-9 with MEDI-528 may affect EIB (13). However, a statistical analysis could not be performed due to the limited small sample size. In order to further investigate whether anti-IL-9 monoclonal Ab has any clinical benefits in patients with asthma, a phase IIb study was performed (14). The double blind, multicenter, parallel-group study enrolled 329 adults randomized (1:1:1:1) to subcutaneous placebo or MEDI-528 (30, 100 and 300 mg) every 2 weeks for 24 weeks, in addition to their usual asthma medications. Failed to reach its primary endpoint, the results revealed that the addition of MEDI-528 to existing asthma controller medications was not associated with any improvement in the Asthma Control Questionnaire-6 scores, asthma exacerbation rates or FEV1 values. This observation may be surprising at the first

Table II. Targeting IL-9 in murine asthma models.

Model	Antigen challenge	Treatment	Results	Reference
B6D2F1	OVA	Anti-IL-9 mAb (200 μg/mouse, i.p.) was given 2 h before sensitization on day 1, 5, 9, and 12. The last dose (on day 12) was given 2 h before antigen challenge.	Treatment with anti-mIL-9 Ab significantly reduced pulmonary eosinophilia, serum IgE levels, goblet cell hyperplasia, airway epithelial damage, and AHR, but had no effect on IL-4, IL-5, and IL-13 mRNA levels in the lungs.	8
BALB/c	OVA	Anti-IL-9 mAb (20 μg/mouse, i.v.) was given 30 min before OVA provocation.	Treatment with anti-mIL-9 Ab significantly prevented AHR. Blockade of IL-9 reduced the numbers of eosinophils and lymphocytes and the concentrations of IL-4, IL-5, and IL-13 in BALF. Macrophage-derived cytokine expression in the airways was also decreased by IL-9 blockade.	9
BALB/c	OVA	Anti-IL-9 mAb (250 μg/mouse, i.p.) was administered 30 min twice during the first week (days 19 and 23) and once weekly thereafter.	Treatment with anti-mIL-9 Ab attenuates MC numbers in the lung, airway remodeling, and is associated with decreased expression of VEGF, FGF-2, and TGFβ.	10
BALB/c	OVA	Anti-IL-9 mAb (100 μg/mouse, i.p.) was given once a week during the OVA challenge.	Numbers of eosinophils, neutrophils, B cells, mast cells, and Th17 cells decreased after administration of anti-IL-9 Ab. Total IgE, IL-5, IL-9, and IL-17 levels were also lower in the anti-IL-9 group.	11
C57BL/6	/	5 μg/mouse of rmIL-9 in BSA and saline solution (20 μl) were intratracheal instilled once each day to B6 mice for 10 days.	Murine trachea insertion of mIL-9 increased eosinophils in the BAL and significantly elevated serum total IgE. IL-9 was also found to induce IL-5Rα *in vivo* and *in vitro*.	46
C57BL/6	/	5 μg/mouse of rmIL-9 in BSA and saline solution (20 μl) were intratracheal instilled once each day to B6 mice for 9 days.	Murine trachea tissue exhibited muc5ac mRNA upregulation and increased numbers of periodic acid Schiff/Alcian blue-positive mucous cells.	47

IL, interleukin; Ab, antibody; OVA, ovalbumin; IgE, immunoglobulin E; AHR, airway hyperresponsiveness; CTGF, connective tissue growth. factor; BALF, bronchoalveolar lavage fluid; VEGF, vascular endothelial growth factor; FGF-2, fibroblast growth factor 2; TGFβ, transforming growth factor beta; BSA, bovine serum albumin.

glance, since recent results obtained from phase IIa clinical studies, as well as earlier observations in mouse models indicated a potential role in asthma. The main reason for this discrepancy may be the influence of asthma subtype and the heterogeneous group of patients presented here and enrolled in the other studies. Thus, it is not surprising that the heterogeneous nature of asthma has received increasing attention recently, as identification of potential subgroups or personal characteristics is likely to be the primary determinant of the efficacy with therapeutics such as MEDI-528.

7. Conclusion

Since it was initially described in the late 1980s, major advances regarding the biology of IL-9 have been achieved. Data from both murine models and clinical trials are now accumulating to support the involvement of IL-9/IL-9R in asthma. Yet, the results from the clinical trials in humans with asthma reflect the heterogeneity of this disease. Therefore, the next challenge before developing successful therapies will be the detailed identification of a potential subphenotype of human asthma. Finally, the determination of whether the asthma phenotype and its underlying mechanisms will allow researchers to devise personalized and phenotype-specific therapies for asthma, remains to be investigated.

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