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A Brief History of IL-9

Ritobrata Goswami and Mark H. Kaplan

IL-9 was first described in the late 1980s as a member of a growing number of cytokines that had pleiotropic functions in the immune system. Although many biological functions have been attributed to IL-9, it remains an understudied cytokine. A resurgence of interest in IL-9 has been spurred by recent work demonstrating a role for IL-9 in regulating inflammatory immunity and defining the transcription factors that activate the Il9 gene in cells that most efficiently produce IL-9. In this review, we summarize the characterization of IL-9 biological activities, highlight roles for the cytokine that are clearly defined, and outline questions regarding IL-9 functions that still require further exploration. The Journal of Immunology, 2011, 186: 3283–3288.

Discovery and cloning of IL-9

IL-9 was first purified and characterized as a T cell and mast cell growth factor respectively termed P40, based on m.w., or mast cell growth-enhancing activity (1, 2). The cloning and complete amino acid sequencing of P40 revealed that it is structurally distinct from other T cell growth factors (3, 4) and was renamed IL-9 based on biological effects on both myeloid and lymphoid cells. A 14-kDa peptide was predicted from the cloned sequence encoding a 144-aa protein, including leader sequence. The mouse Il9 gene is located on chromosome 13, whereas the human IL9 gene is located in a syntenic region on chromosome 5 (5). IL-9 is 3.2 megabases telomeric from the IL5/IL13/IL4 loci.

IL-9-secreting cells

The major source of IL-9 is T lymphocytes. Initially described as T cell growth factor III, IL-9 is produced by long-term T cell lines, Ag-specific T cell lines, and naïve murine T cells (6). However, it was unclear if there were specialized cells programmed for IL-9 production as was being established at the time for other cytokines such as IL-4 and IFN-γ in Th2 and Th1 cells, respectively.

IL-9 production was first associated with the Th2 phenotype, and many of the preliminary functions of IL-9 were tested in models of Th2-associated immunity. The expression of IL-9 in T cells isolated from Leishmania major-infected BALB/c mice, which generate a Th2-biased immune response, provided the initial basis for defining IL-9 as a Th2 cytokine (7). However, other Th subsets also appear to have the potential for IL-9 production. Th17 cells, which are defined by secretion of IL-17A and IL-17F, may also secrete IL-9 in vitro and ex vivo (8, 9). Human Th17 cells can secrete IL-9, and long-term Th17 cultures have the ability to coexpress IL-17A and IL-9 (10). In contrast, IL-23, a cytokine required for maintenance of the IL-17–secreting phenotype, has inhibitory effects on IL-9 production (8, 11). Regulatory T cells (Tregs) may also produce IL-9. A study linking mast cells to peripheral tolerance demonstrated that natural Tregs and inducible Tregs, both Foxp3+ populations, secrete IL-9 (12). There is conflicting evidence regarding production of IL-9 from human Tregs (10, 13).

Mounting evidence suggests that there may be a specialized subset of T cells dedicated to producing IL-9, in which Il9 is regulated by a number of cytokines and transcription factors (Fig. 1). More than 15 y ago, TGF-β and IL-4 were shown to enhance IL-9 production from activated T cells (14). 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 and showed diminished expression of other lineage-specific cytokines and transcription factors (15, 16). The ability of IL-4 to inhibit expression of Foxp3 in cultures containing TGF-β that induce inducible Treg generation and to promote an IL-9–secreting phenotype was dependent on STAT6 (15, 16). Similarly, GATA3 was required for the generation of IL-9–secreting cells (15), suggesting that Th2 cells, and the IL-9–secreting population termed Th9 cells, have shared factors in their development. Human T cells also acquire IL-9–secreting potential when cultured with TGF-β and IL-4 (10, 13, 17–19). The stability of the IL-9–secreting phenotype is still not well described, although studies suggest that Th9 cells can be maintained, but retain plasticity to acquire additional cytokine-secreting potential (20).

Recently, two transcription factors were reported to bind directly to the Il9 gene and were required for the development of IL-9–secreting cells. PU.1 is an ETS family transcription factor known to have a repressive effect on the production of Th2 cytokines (21, 22). Ectopic expression of PU.1 not only
In T cells, the most efficient priming of IL-9 production occurs in response to a combination of TGF-β and IL-4. PU.1 may be downstream of the TGF-β signal, and STAT6 and GATA3, both required for the development of IL-9–secreting T cells, are downstream of IL-4. IRF4 expression may be regulated by both signals. It is not known if STAT6 or GATA3 binds the IL9 gene directly. PU.1 and IRF4 bind the Il9 promoter directly in Th9 cells. AP-1 and NF-κB bind the promoter in response to TLR stimulation in mast cells and are likely downstream of IL-1 and IL-25 signaling in T cells.

represses Th2 cytokines, but also induces IL-9 production (17). PU.1-deficient T cells have greatly diminished IL-9 production compared with wild-type cells when cultured in the presence of TGF-β and IL-4. PU.1 binds directly to the Il9 gene, and histone modifications associated with the Th9 phenotype are dependent upon PU.1. PU.1 is also important for IL-9 production in human T cells as PU.1-specific small interfering RNA results in impaired IL-9 production by human T cells. Importantly, PU.1 is expressed in greater amounts in cells cultured under Th9 conditions compared with Th2 cells, suggesting that PU.1 is a critical factor in diverting Th2 cells into an IL-9–secreting lineage (17). IFN regulatory factor 4 (IRF4) was also shown to be required for Th9 generation (18). Like PU.1, and possibly in concert with PU.1 because IRF4 was originally identified as a PU.1-interacting protein, IRF4 bound the Il9 gene directly (18). Moreover, IRF4 expression is correlated with both human and mouse Th9 differentiation and is induced by cytokines that promote IL-9 production including IL-4, IL-2, and TGF-β (18). As IRF4 is also required for Th2 and Th17 development (23–25), this transcription factor plays an important role in the cytokine-secreting potential of several Th subsets.

Mast cells also produce IL-9 in response to LPS and IL-1, correlating with the presence of NF-κB binding sites in the Il9 promoter that mediate gene activation (26–28). GATA1 in mast cells promotes IL-9 production and Il9 promoter activation in a manner that is dependent upon p38 MAPK (29). The relative amounts of IL-9 produced by mast cells and T cells, as well as the scenarios in which each cell may contribute to IL-9 production in vivo, have not been determined.

**Amplifiers of IL-9 production**

TGF-β and IL-4 are potent cytokines in promoting the generation of IL-9–secreting cells. Indeed, the ability of TGF-β to promote IL-9 expression is likely responsible for the ability of Treg and Th17 cultures to secrete IL-9 (8, 9) and may have similar effects on both naive and memory cells (10, 13). In addition, other cytokines and signaling pathways are able to modulate IL-9 production. IL-2 promotes, whereas IFN-γ inhibits IL-9 production (14, 30). IL-25 can enhance IL-9 production by Th9 cells and promote IL-9 production in vivo (31). These observations are consistent with the ability of NF-κB signaling to promote transcription from the IL-9 promoter (28). Addition of other proinflammatory cytokines to human Th9 cultures, such as type I IFNs, IL-1β, IL-6, IL-10, IL-12, and IL-21, may also enhance IL-9 production (10, 13, 19, 32). The ability of type I IFNs to promote IL-9 production is dependent upon IFN-induced IL-21 expression (19). Whether the effects of these accessory cytokines are dependent upon other STAT proteins or other transcription factors that activate Il9 transcription such as NF-κB or AP-1 (33) has not yet been determined. TCR signaling also induces IL-9, and though cyclosporine A inhibits IL-9 production, suggesting a role for NFAT proteins (7), the TCR-induced signals that lead to expression of Il9 are not characterized.

**The IL-9R: signaling and expression**

The IL-9R has two subunits: the α-chain (IL-9Rα) and the common γ-chain receptor shared by other cytokines including IL-2, IL-4, and IL-7 (34, 35). The human IL-9R gene contains 11 exons and encodes a 522-aa protein, whereas the mouse receptor contains 468 aa. The IL-9Rα is a member of the hematopoietin superfamily based on the presence of WXXWS motif in the extracellular domain and the Box1 and Box2 motifs in the intracellular domain. IL-9–mediated signal transduction results in the activation of STAT1, STAT3, and STAT5 (36). A single tyrosine residue (Tyr407) in the IL-9Rα is phosphorylated after ligand binding to the receptor and activation of associated Jak1, and mutation of this residue demonstrates that it is required for IL-9–dependent responses (37). The Box1 intracellular domain between aa 338 and 422, including a YLPQ motif, is critical for IL-9–induced cell growth, activation of STAT3, and induced gene expression (38). IL-9R signaling also activates MAPK and insulin receptor substrate–PI3K pathway (37, 39, 40), though the physiological requirement for these pathways in primary cells has not been well documented.

As expected from its initial identification as a T cell growth factor, IL-9R is expressed on T cell lines and on effector T cells but not naive T cells (41, 42). Among the Th cell subsets, IL-9R has highest expression in Th2 and Th17 cells (9). In asthma patients, IL-9R is found on mast cells and polymorphonuclear cells in the lung (43, 44). Based on the responsiveness of airway epithelial cells and smooth muscle cells, IL-9R is expressed on nonhematopoietic cells as well. Because γc is unlikely to be expressed in these cells, the exact composition of the receptor on nonhematopoietic cells is not clearly defined.

**Cell type-specific functions of IL-9**

As suggested by the patterns of receptor expression, IL-9 has biological effects on a number of distinct cell types (Fig. 2). Beyond the first description as T cell or mast cell growth factor, IL-9 may affect other immune cells, as well as resident tissue cells that contribute to the development of inflammation.

**T cells.** Although IL-9 was originally cloned as a T cell growth factor, its function was more restricted than cytokines with similar functions, like IL-2 and IL-4. IL-9 could promote T cell growth, but it functioned preferentially on CD4+
Airway epithelial cells. Transgenic expression of IL-9 in the lung results in changes in gene expression in airway epithelial cells including goblet cell metaplasia (62). Many of the effects of IL-9 in the lung are thought to occur due to indirect effects of IL-13 (46, 63–66). In this scenario, IL-9 promotes allergic inflammation through effects on hematopoietic cells that then produce IL-13 to have direct effects on airway epithelium (64). However, IL-9 can have direct effects on human primary airway epithelial cells and cells lines including the direct induction of mucus genes (67–70). Thus, IL-9 may have both direct and indirect effects in the airway.

Airway smooth muscle cells. In the lung environment, IL-9 also acts on airway smooth muscle cells. Human airway smooth muscle cells express both IL-9R (71), and IL-9 can potentiate the ERK-dependent release of CCL11 and IL-8 from these cells (71, 72). IL-9–mediated CCL11 expression in primary smooth muscle cells is dependent upon STAT3 but not STAT6 (73). IL-9–activated STAT3 binds directly to the CCL11 promoter (73).

IL-9–dependent regulation of inflammation

IL-9 demonstrates proinflammatory activity in several mouse models of inflammation. Transgenic expression of IL-9 in the lung results in allergic inflammation that is at least partially dependent on the presence of Th2 cytokines (46, 62). IL-25 also promotes allergic inflammation through an IL-9–dependent mechanism (31). Although allergic inflammation can develop in Il9−/− mice using a standard sensitization-challenge model (74), four reports have demonstrated that blockade of IL-9 in a similar model results in decreased allergic inflammation (17, 31, 75, 76). Similarly, blocking IL-9 in a chronic model of lung inflammation inhibits mastocytosis and airway remodeling (44). Moreover, Th9-polarized allergen-specific T cells can promote allergic inflammation that is blocked by anti–IL-9 (18). The reason for the discrepancy between germline disruption of Il9 and acute blockade by anti–IL-9 is not entirely clear, but the immune system may be able to compensate for the germline loss of cytokine. A role for IL-9 in the development of allergic pulmonary inflammation is further supported by the requirement for the T cell expression of IL-9–promoting transcription factors PU.1 and IRF4 in this process (17, 18). In mice with PU.1-deficient T cells, Th2 development is normal, stressing the importance of IL-9–secreting cells for the development of inflammation even in the presence of allergen-specific Th2 responses (17).

IL-9 mediates allergic inflammation in tissues other than the lung. IL-9 provided either by transgene or injection increases susceptibility to passive or active systemic anaphylaxis (77). However, systemic anaphylaxis occurred in Il9−/− and Il9r−/− mice, suggesting that IL-9 is not absolutely required for anaphylaxis (77, 78). In contrast, deficiency in IL-9 or IL-9R attenuates intestinal anaphylaxis, and transgenic expression of IL-9 in the intestine resulted in local mastocytosis and increased susceptibility to intestinal anaphylaxis (78, 79). Thus, IL-9 may be required for anaphylaxis caused by allergen challenge at mucosal surfaces but not parenterally administered allergen.

IL-9 provides a protective role in immunity to intestinal parasites. In mice with transgenic expression of IL-9, there is increased clearance of worms following infection with Trichuris muris and Trichinella spiralis (80, 81). Similarly, adoptive transfer of IL-9 transgenic bone marrow–derived dendritic cells increases immunity to T. spiralis, whereas anti–IL-9 blocks T. muris immunity (82, 83). Blocking TGF-β signaling, which inhibits the development of Th9 cells, also results in diminished immunity to T. muris (16). In contrast, IL-9–deficient mice were able to control Giardia lamblia and Nippostrongylus brasiliensis infection, and neutralization of IL-9 resulted in enhanced immunity to L. major, owing to blockade of Th2 immunity (52, 84, 85). IL-9 was also not...
required for granuloma formation following injection with Schistosoma mansoni eggs, though it was important for mastocytosis and goblet cell metaplasia in this model (52). Yet, IL-4 is able to promote intestinal parasite immunity in the absence of IL-9 and other Th2 cytokines (86).

There is also evidence that IL-9 plays a role in regulating immunity to infectious disease. IL-9 negatively impacts clearance of respiratory syncytial virus and neutralization of IL-9 may be therapeutic (87). Symptoms of septic shock may also be attenuated by IL-9, which may be through several mechanisms, including suppression of inflammatory cytokines and the respiratory burst in monocytes (88, 89).

Although IL-9 is involved in what are classically thought to be Th2 responses, there are conflicting data on how it may be involved in autoimmune inflammation. Adoptive transfer of Th9-polarized cells can promote the development of experimental autoimmune encephalomyelitis (EAE) and experimental autoimmune uveitis, though the inflammation that develops is distinct from Th1- or Th17-mediated immunity (11, 20). In two reports, IL-9Rα deficiency or treatment with anti–IL-9 were either partially protective or resulted in delayed development of EAE (9, 90). Results from another report demonstrated increased severity of EAE in Il9−/− mice that was correlated with an effect of IL-9 on Treg function (8). The anti-inflammatory role proposed in the latter report is consistent with a described role for IL-9 in promoting mast cell-dependent tolerance to transplanted allografts and nephritis (12, 91). IL-9 certainly has effects in the EAE models on T and non-T cells, and the effect of blocking IL-9/IL-9R may depend upon the cells present in the microenvironment (9).

IL-9: from the bench to the bedside

Parallel to in vitro studies and experiments in mouse models, IL-9 is correlated with allergic inflammation in patients. IL-9 expression is increased in lungs of asthmatic patients (92, 93), and IL-9R expression is found in the lungs of asthmatic individuals but not healthy controls (94). Seasonal ragweed pollen enhances IL-9 production from PBMCs of allergic patients (95). Because of the presence of IL-9 in allergic inflammation, blocking Abs to IL-9 are being developed as a therapy for atopic disease (96).

Some genetic links between disease and IL-9 signaling have also been identified. Single nucleotide polymorphisms in the IL9R gene, located on the X chromosome, were protective for wheezing in boys, but not girls, and provided a modest protective effect for allergen sensitization (97). IL9 polymorphisms are also linked to sex-restricted differences in lung function, allergen sensitization, IgE levels, and the severity of respiratory syncytial virus infection (98, 99). An IL9R polymorphism (rs3093457) has been linked to rheumatoid arthritis (100). Together, these results suggest that changes in IL9 or IL9R expression can impact human disease.

Conclusions

IL-9 is a pleiotropic cytokine that has documented effects on lymphocytes, mast cells, and resident lung cells (Fig. 2). It has been most frequently associated with allergic inflammation and immunity to extracellular parasites, although developing literature has demonstrated a role for IL-9 or IL-9–responsive cells in Th1/Th17-mediated inflammation and in Treg responses. The factors required for IL-9 production in T cells are only beginning to be elucidated and require the integration of signals from multiple cytokines (Fig. 1). The next challenges will be to develop a more detailed understanding of IL9 regulation and define the relevant IL-9 target cells in various inflammatory diseases. Although IL-9 has been understudied, recent advances in defining IL-9 biology will likely promote greater attention.

Disclosures

The authors have no financial conflicts of interest.

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