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The world according to IL-9

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Abstract

Among the cytokines regulating immune cells, interleukin 9 (IL-9) has gained considerable attention for its ability to act on multiple cell types as a regulator of beneficial and pathologic immune responses. Yet, it is still not clearly defined how IL-9 impacts immune responses. IL-9 demonstrates a remarkable degree of tissue-specific functionality and has cellular sources that vary by tissue site and the context of the inflammatory milieu. Here, we provide perspective to summarize the biological activities of IL-9 and highlight cell type-specific roles in the immune pathogenesis of diseases. This perspective will be important in defining the diseases where targeting IL-9 as a therapeutic strategy would be beneficial, and where it has the potential to complicate clinical outcomes.

Introduction to IL-9 responsiveness

The function of interleukin 9 (IL-9) has been a puzzle almost since its discovery. While it was first associated with type 2 immune responses, subsequently identified roles for IL-9 in autoimmunity and tumor immunity have prevented it from being neatly categorized as a component of type 1, type 2, or type 3 (type 17) immunity. Moreover, there are no immune responses yet identified where IL-9 is the predominant cytokine being produced, first suggesting that there are no ‘type 9’ immune responses, and second supporting a paradigm where the outcomes of IL-9 stimulation are impacted by responses to other factors in the environment. While there are many beneficial and pathologic immune responses where IL-9 is critical, the outcome of the response to IL-9 varies with how the responding cell integrates the cytokine milieu.

IL-9, initially named P40, was first identified as a T cell and mast cell growth factor, functions that have now been demonstrated *in vivo* in multiple models (1). The source of IL-9 was initially associated with T cells, but mast cells, neutrophils, basophils, eosinophils, and innate lymphoid cells can also produce varying amounts of IL-9 (1). Cytokines greatly contribute to regulating IL-9 expression. *In vivo* approaches using neutralizing antibodies against IL-4, IL-33, and TGFβ effectively diminish IL-9 synthesis, implying a regulatory role in IL-9 production (2–4). *In vitro* cell culture systems of mouse and human cells confirmed that IL-4, IL-33, and TGFβ directly induce IL-9 production in CD4+ T cells and mast cells (3–7). In addition, IL-9 expression in CD4+ T cells can be further enhanced by

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IL-1, IL-2, IL-25, or Activin A; while IFNγ or IL-23 can diminish IL-9 expression levels (5, 8–10). Several TNFSF members also induce IL-9 production (OX40, TL1A, GITRL) (11–13). At the molecular level, *Il9* gene expression is promoted by transcription factors that include PU.1 (14), IRF4 (15), BATF (16), Smad (7), Gcn5 (17), IRF8 (18), NF-κB (12, 13), ETV5(19), ERG (20), STAT5 (21), STAT6 (17), and other factors (22), acting through several cis-regulatory elements (23–25), Taken together, IL-9 and IL-9-producing cells can be influenced by the cytokine milieu to enhance or reduce IL-9 production by targeting the *Il9* gene through a multitude of transcription factors. This is likely important as it is still not clear if the source of IL-9 is a factor in the outcome of the response. It is possible and perhaps likely that the IL-9-producing cell itself secretes other cytokines or expresses other surface molecules that impact the outcome in the responding cell.

IL-9 utilizes a heterodimeric receptor consisting of IL-9Rα and common γ chain to allow for the activation of intracellular signaling cascades. IL-9R is expressed on hematopoietic cells including T cells, B cells, myeloid cells, mast cells, and epithelial cells (26). IL-9Rα expression is variable, with some cells, mast cells for example, expressing abundant surface receptor regardless of activation state, while other cells express receptor in a differentiation-specific manner. IL-9Rα is also expressed on non-hematopoietic cells including airway and intestinal epithelial cells, smooth muscle cells, and keratinocytes (27–29). The lack of γc on non-hematopoietic cells suggests that there could be another receptor complex which would provide a structural basis to further diversify the response to IL-9.

An understanding of the secreting cell, the responding cell, and the context for receiving the IL-9 signal begins to provide the foundation for determining IL-9 function. In this review we will dissect the cell-type specific functions of IL-9 in allergic inflammation, tumor immunity, autoimmunity, and infection. We will further highlight where there are direct effects of IL-9 on a particular cell type with the goal of providing a picture of the cellular landscape from the perspective of IL-9.

Lymphocytes

As noted above, IL-9 was originally identified as a T cell growth factor based on *in vitro* experiments (30). There are numerous models where IL-9 promotes inflammation or tumor immunity where the effect on T cell expansion can be observed following neutralization of IL-9 (5, 6, 31, 32). In a mouse model of allergic asthma, the absence of IL-9/IL-9R signaling significantly reduced CD4+ T cell numbers, demonstrating that IL-9 can affect T lymphocytes (31, 33). However, despite mouse and human T cells having abundant expression of *Il9r/IL9R* (6, 29), whether these observations are due to direct effects of IL-9 on T cells is still unclear.

IL-9 also impacts CD4+ T cell responses by altering their cytokine profile and their ability to respond to the environment (Figure 1). CD4+ Th17 cells are key IL-9 responders in a mouse model of experimental autoimmune encephalomyelitis (EAE); however, *the exact function of IL-9 in this disease remains controversial*. In some studies, mice deficient in IL-9 signaling ($Il9^{-/-}$ and $Il9r^{-/-}$) were resistant to the induction of EAE, which correlated with reduced Th17 cells, central nervous system (CNS) cellular infiltration, and less production

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of the inflammatory cytokine IL-17, IFNγ, and TNFα (6, 34, 35). In agreement with this, neutralizing IL-9 antibody effectively suppressed the incidence and severity of EAE (34). Specifically, blockade of IL-9 led to reduced C-C chemokine receptor expression of CCR2, CCR5, and CCR6 which could contribute to the reduced T cell migration in the CNS and suppressed myelin-specific Th1 and Th17 differentiation (5, 6). The ability of IL-9 to promote Th17 development *in vitro* (5) suggests that the effects *in vivo* on T cells are indirect and an effect of diminished inflammation. In contrast, the decrease in Treg frequency observed with anti-IL-9 mAb treatment support the role of IL-9 on Tregs described in Elyaman *et al* (5). Thus, in addition to its role of suppressing Th1 and Th17 induction, blockade of IL-9 can reduce the suppressive capacity of Tregs thereby allowing for T cell proliferation and effector function to support EAE induction (5). Under certain conditions, IL-9 may play contrasting roles in disease development that may be due to cell type-specific effects. Moreover, studies utilizing different methodologies such as monoclonal or polyclonal antibodies, dosage of rIL-9 or anti-IL-9 antibodies, or varying timing and routes of administration could lead to differences in experimental outcomes. These studies highlight the importance of understanding the regulation of IL-9 signaling in development of inflammatory diseases. Accordingly, studies are still needed to investigate the direct effects that IL-9 exerts on CD4+ T cell subsets.

The effects of IL-9 on CD8+ T cells have been best characterized on cytotoxic CD8+ T cells (CTL) in anti-tumor responses against breast cancer, colorectal cancer, and melanoma. IL-9 can act as an immunostimulatory cytokine to modulate CTL numbers and cytolytic functions. Notably, IL-9R expression on CD8+ T cells correlates with lower expression of the exhaustion markers PD-1 and TIM-3 when examined *ex vivo* in breast cancer patients and healthy controls, supporting the role for IL-9 in enhancing CTL antitumor responses (36). Moreover, exogenous IL-9 can increase expression of IL-9R in CD8+ T cells and changes the balance of cytokine production, lowering IFNγ and increasing IL-2 and IL-17 (36). This effect may have an autocrine function to promote proliferation of CD8+ T cells. Indeed, CD8+ T cells isolated from patient colorectal cancer tissues can be expanded in the presence of IL-9 (37). The overall decrease in inhibitory receptors may suggest that these CD8+ T cells are less susceptible to immune suppressive mechanisms in tumor microenvironment, thus allowing for greater effector function by enhancing cytokine expression. *In vivo* IL-9 blockade promoted melanoma progression in a murine melanoma model; moreover, *in vitro* IL-9 blockade in human PBMCs led to diminished cytolytic molecules, granzyme B and perforin, in CD8+ T cells (38). This is consistent with a study using a glucocorticoid-induced tumor necrosis factor receptor-related protein agonist that can trigger IL-9 expression transiently at the early stages of tumor progression in a murine model of CT26 colon cancer. In this model, they showed that tumor-bearing mice treated with IL-9 neutralizing antibody had reduced granzyme B, TNFα, IFNγ, and CD107a expression in tumor-specific CD8+ T cells (13). These data indicate that IL-9 can contribute to initiating and maintaining antitumor immunity by modulating CD8+ T cell cytotoxicity (39) and this likely contributes to the ability of IL-9-secreting CD8+ T cells to promote highly efficient anti-tumor responses (Figure 1).

IL-9R is expressed in resting and activated germinal center B cells (40). Overexpression of IL-9 can promote B cell responses by enhancing baseline and antigen-specific antibody

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production of IgG and IgE (40, 41) (Figure 1). IL-9 induces STAT3 and STAT5 activation in B cells to potentiate IgG and IgE production (42) (Figure 1). Consistent with this, in an allergic inflammation model, deficiency in IL-9R reduced CD40-dependent proliferation and plasma cell differentiation of B memory cells thus severely diminishing recall antibody production (43) (Figure 1).

IL-9R is dispensable for type 2 innate lymphoid cell (ILC2) maintenance in naïve mice but required for survival of activated ILC2 in a mouse model of *Nippostrongylus brasiliensis* lung infection (44). In *N. brasiliensis* infection, ILC2 accumulation, amphiregulin, IL-5 and IL-13 production is dependent upon IL-9R signaling (44, 45) (Figure 1). IL-9 enhances BCL3-dependent anti-apoptotic protein expression to enhance ILC2 survival (44, 46). Patients with mastocytosis have fewer IL-9R+ ILC2 which correlated with lower plasma IL-9, higher IgE levels, and decreased circulating Tregs (47) (Figure 1). CD25+ ILC stimulated with IL-9 exhibited enhanced IL-5, IL-6, and IL-13 production compared to unstimulated controls, and in parallel neutralizing IL-9 antibodies led to diminished cytokine production but did not impact ILC number (45) (Figure 1). Furthermore, in the lung tissues of house dust mite (HDM)-challenged mice, anti-IL-9 neutralizing antibodies led to diminished ILC2 numbers that are likely similar to the reduced ILC2 proliferation also observed in $Il9^{-/-}$ mice, and changes in gene expression of cytokines and other effectors (31, 33). Together, reports suggest IL-9 directly impacts ILC expansion and effector function.

IL-9R is expressed in a number of lymphoma cell lines: diffuse large-B cell lymphoma, mantle cell lymphoma, and human acute T cell leukemia Jurkat cell lines (48). *In vitro* studies showed that IL-9 directly induced proliferation and inhibited apoptosis in lymphoma cell lines (48, 49). In support of this, *in vivo* studies demonstrate that blockade of IL-9 decreases lymphoma cell proliferation (50, 51) (Figure 1).

Mast cells

Initial studies characterizing IL-9 function identified mast cells as one of the main targets (52). IL-9R+ mucosal (mouse) or tryptase-expressing (human) mast cells account for most IL-9 responsive mast cells within murine or human tissue at homeostasis. Mast cell phenotypes are likely influenced by signals from the tissue microenvironment, with IL-9 being highly expressed in mucosal tissues.

In murine models of allergic airway inflammation, blockade of IL-9 led to diminished lung mast cell numbers and impaired mast cell activation and decreased expression of the profibrotic mediators, TGFβ, VEGF, and FGF2 (53, 54). Similarly, blockade of IL-9 in a mouse model of renal disease, nephrotoxic serum nephritis, effectively reduced mast cell numbers in the kidney-draining lymph nodes leading to increased disease (55). These findings demonstrate that the effects of IL-9 on mast cells are tissue-specific and is strictly regulated in various diseases.

Subsequent work in mice deficient for either IL-9 or IL-9Rα showed defective expansion mast cells in the bone marrow, peritoneal cavity, lung, and intestines in response to infection or following the induction of allergic airway inflammation (23, 56–58). As previously noted,

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IL-9 has been shown to exert both helpful and harmful effects, depending on the context. In situations where mast cells are helpful, such as in host defense against infection, deficiency in IL-9 can be detrimental to clearance of infection. This is observed in a mouse model of a parasitic infection with *Strongyloides ratti (S. ratti*) where IL-9R-deficient mice exhibited decreased mast cell activation and degranulation which correlated with greater parasite burden (59) (Figure 1). When IL-9 is overexpressed using IL-9-transgenic mice or treatment with rIL-9, mice have enhanced mucosal mast cell responses that promote worm expulsion (60, 61). However, other studies have found that IL-9 can exacerbate mast cell-dependent allergic inflammation. In models of allergic diseases, mice deficient in IL-9 signaling had fewer mast cell numbers and mast cell protease (MCPT1) expression (62–64). Using a food allergy or allergic airway inflammation model, mice deficient in IL-9 signaling were protected against antigen-induced systemic anaphylaxis and airway hyperresponsiveness, demonstrating that IL-9 can contribute to outcomes of allergic disease by promoting mast cell expansion and enhancing mast cell responses (62–64) (Figure 1). Since IL-9 is highly expressed in mucosal sites and IL-9 responsive mast cells are poised in mucosal tissues, it is likely that the microbiome can contribute to the development of inflammatory diseases by acting through mast cells (65–67). It is still not clear how the IL-9/mast cell axis impacts local microbiome.

In vitro evidence suggests that IL-9 has direct effects on mast cells. These studies revealed that IL-9 can induce VEGF secretion in human mast cells (68), and IL-2 and TGFβ1 expression in murine mast cells (56, 69) (Figure 1). Since these secreted factors could have pro-inflammatory downstream effects, it is possible that IL-9 can also indirectly contribute to exacerbating inflammation. Although the role of mast cells in tumor immunity has been extensively studied, exactly how IL-9 modulates mast cell anti-tumor responses remains to be clearly defined.

Antigen Presenting Cells (Monocytes, Macrophages, and Dendritic cells)

Myeloid cells other than mast cells have not been classically thought of as important IL-9 responding cells. Yet, IL-9R is easily detected in monocytes, macrophages, bone marrow-derived dendritic cells (BMDC), myeloid dendritic cells, and plasmacytoid dendritic cells (70, 71). Notably, in the CNS, cells committed to resolve inflammation such as nonclassical monocytes and plasmacytoid dendritic cells express lower levels of IL-9R compared to other monocytes or myeloid dendritic cells. The ability of IL-9 to promote inflammation by altering myeloid cell function is likely linked to environmental impacts on IL-9R expression.

There are numerous studies where IL-9 can have direct effects on isolated myeloid cells *in vitro* (70–72). For instance, human macrophages stimulated with IL-9 express reduced inflammatory markers CD45, CD68, CD14, and CD11b, suggesting that IL-9 can regulate macrophage migration and phagocytic activity (70) (Figure 1). Similarly, LPS-stimulated monocytes and alveolar macrophages stimulated with IL-9 obtained an anti-inflammatory phenotype with enhanced TGFβ production and diminished TNF-α and IL-10 release (71, 72) (Figure 1). This anti-inflammatory function of IL-9 on monocytes and macrophages supports its relevance as a therapeutic target to dampen severe inflammation, specially observed in autoimmune diseases such as multiple sclerosis.

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Many of these *in vitro* responses can be recapitulated *in vivo* when IL-9 activity is blocked. In a mouse model of EAE, blockade of IL-9 using anti-IL-9 neutralizing antibodies attenuated EAE inflammation by decreasing IL-6 producing macrophage in the CNS and regional lymph node (6). Similarly, neutralizing anti-IL-9 antibodies inhibited the production of reactive oxygen intermediated in activated human blood monocytes and alveolar macrophages, consequently inhibiting oxidative burst responses which may be required for regulating lung tissue injury (71–73). In cancer, blockade of IL-9 downregulated co-stimulatory and MHCII molecules and decreased DC cross-presentation capacity thereby diminishing tumor-specific CTL responses (13, 74) (Figure 1).

In the absence of an IL-9R conditional allele, some of the best experiments to define cell type specific effects have used adoptive transfer to interrogate function. As we've described earlier, IL-9 likely regulates neuroinflammation by dampening inflammatory myeloid cells. Indeed, adoptive transfer of *Il9r*$^{-/-}$ dendritic cells to wild type (WT) mice led to exacerbated autoimmune neuroinflammation disease development that correlated with increased GM-CSF-producing T cells in the CNS (75) (Figure 1). In contrast, in a chronic model of airway inflammation, IL-9R deficiency in *Il9r*$^{-/-}$ mice protects against allergic inflammation, suggesting that IL-9 promotes pro-inflammatory functions in lung macrophages. Adoptive transfer of interstitial macrophages into *Il9r*$^{-/-}$ mice led to greater inflammation observed with higher lung cell number and eosinophil infiltration into the allergic lung (76). Moreover, the transfer of wild type macrophages to *Il9r*$^{-/-}$ mice was able to rescue the development of airway inflammation that was lost in the absence of IL-9 signaling, further supporting the pro-inflammatory role of IL-9 on macrophages (76). IL-9 also impacted the ability of monocytes to be recruited to the lung (76). Among IL-9 regulated genes, arginase 1 (*ARG1*) in macrophages was critical for IL-9-mediated inflammation (76) (Figure 1). Parallel observations were made in a lung cancer model where IL-9 signaling in macrophages promoted the development of tumors in the lung (77). This was in contrast to a B16 lung cancer model where the tumor was engineered to overexpress IL-9, resulting in increased CD80 and CD86 expression in lung, spleen, and peritoneal-derived macrophages, driving M1 macrophage polarization which enhances anti-tumor cytotoxicity (78) (Figure 1). These disparate effects may be due to gating of differing populations of macrophages or ectopic effects of IL-9 in the tissue microenvironment. Nonetheless, these results highlight the importance of the amount of IL-9 present in the milieu and as noted earlier, the source of IL-9 may impact the outcome of an IL-9 response.

Granulocytes (Neutrophils and Eosinophils)

At steady-state, neutrophils do not respond to IL-9 as they lack surface expression of IL-9Rα (79). Notably, IL-9Rα was detected intracellularly but was not expressed at the surface-level in neutrophils from healthy patients (80). However, during an inflammatory response, the expression of IL-9Rα was variable, but detected in human BAL-derived and PBMC-derived neutrophils, demonstrating that expression of IL-9Rα can be induced and can be regulated by the tissue microenvironment (80). Although the precise mechanism regulating IL-9Rα expression on neutrophils remains to be explored, IL-9 neutralizing antibodies can reduce blood neutrophil cell numbers in a mouse model of allergic airway inflammation in Balb/C mice (81). Functionally, IL-9 can induce the production and release

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of IL-8 in neutrophils, which may function to promote recruitment of other inflammatory cells (80) (Figure 1).

IL-9 is a known enhancer of IL-5-driven airway eosinophilia by promoting eosinophil precursor maturation in the bone marrow (82). IL-9Rα on human eosinophils was detected at the transcriptional and protein level (83, 84). Functionally, IL-9 can enhance IL-5Rα expression thereby enhancing eosinophil development and can inhibit eosinophil apoptosis in a dose-dependent manner (83, 84) (Figure 1). During an acute model of allergic airway inflammation, administering one dose of neutralizing monoclonal anti-IL-9 antibody reduced bone marrow mature eosinophils, but was unable to attenuate the development of eosinophilia in the lung tissue, suggesting that the effects of IL-9 can be tissue-specific and may likely be regulated by IL-9R expression (81). In support of this, *Il9*$^{-/-}$ mice exhibited reduced HDM-induced eosinophil infiltration into the airways compared to WT control mice (33). *It is still unclear if the effects observed in vivo on eosinophils are direct or indirect*.

Non-hematopoietic cells (Epithelial cells, Smooth muscle cells, Keratinocytes, Tumor cells, Glial cells, and Hepatocytes)

Although it has not been well characterized, IL-9 can also act on airway epithelial cells and smooth muscle cells (85–87). Similar to other cell types, IL-9R is not typically expressed on epithelial cells, yet expression of IL-9R can be increased during an inflammatory response (88). Intratracheal administration of IL-9 increased mucus production in the airways shown by Periodic acid–Schiff (PAS)-stained epithelial cells as well as expression of mucin-related genes, *MUC2* and *MUC5AC* (87) (Figure 1). In an allergic model, IL-9 can stimulate airway epithelial and smooth muscle cell chemokine production: CCL2, CCL3, CCL7, CCL11/Eotaxin, and CCL12, which can allow for cellular infiltration of other immune cells into inflamed tissues (85, 89, 90) (Figure 1). Moreover, IL-9 can also regulate intestinal barrier function by inducing claudin-2, a transmembrane protein important for regulating barrier permeability, in intestinal epithelial cells treated with rIL-9 *in vitro* (88) (Figure 1). Subsequent research further exploring the role of IL-9 *in vivo* expanded upon IL-9-dependent and IL-9-independent effects on epithelial cells. In particular, IL-9 can regulate mucus production that is independent of IL-5 and IL-13. Moreover, IL-9-deficient mice (*Il9*$^{-/-}$) are unable to increase goblet cell production despite normal IL-13 expression, indicating that IL-9 can promote goblet cell metaplasia independent of IL-13 (91). This is consistent with research utilizing neutralizing antibodies in which antibody blockade of IL-9, but not IL-5 or IL-13, inhibited mucus production in an allergic airway inflammation model in dogs (86) (Figure 1). Thus, IL-9 can regulate airway epithelial cells and smooth muscle cells; however, *how IL-9 signals downstream of IL-9R requires further exploration*. In addition, the role of IL-9 in epithelial cells and smooth muscle cells at different tissues such as in the trachea, remains to be explored.

IL9R expression is constitutively low in human keratinocytes, but it can be induced during type 2 inflammatory conditions (92, 93). This is best depicted in individuals with atopic dermatitis expressing increased IL-9R expression on epidermal keratinocytes compared to healthy controls (93). These findings demonstrate that keratinocytes are IL-9 responsive and

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suggest that IL-9 can contribute to allergic skin inflammation. Several studies have utilized *in vitro* culture assays and IL-9 neutralizing antibodies to show that IL-9 can regulate keratinocyte expansion, cytokine production, and migration (92–96) (Figure 1). Stimulation of human primary keratinocytes (HPK) with IL-9 enhanced survival by reducing reactive oxygen species-mediated apoptosis, whereas in another report IL-9 induced Ki67 expression to promote HPK proliferation (92, 97) (Figure 1). Furthermore, IL-9 can promote HPK cytokine secretion as observed *in vitro* where IL-9-stimulated HPK exhibited increased IL-8 (CXCL8) production, and vascular endothelial growth factor secretion (93–95) (Figure 1). One recent study has also demonstrated that treatment with rIL-9 can inhibit IFNγ and IL-17A-induced HPK migratory potential by regulating the actomyosin cytoskeleton (96) (Figure 1).

IL-9 acts on neurons to enhance murine newborn neocortex neural survival by inhibiting expression of Bax, a pro-apoptotic molecule (98). IL-9 also induced TGFβ1 expression in cultured neurons that exacerbates the brain lesion development observed in infants with cerebral palsy (69). IL-9R is constitutively expressed on astrocytes (99), and treatment with IL-9 induces CCL20 production which can enhance Th17 migration and contribute to EAE induction (99) (Figure 1). Nonetheless, although other neural cells express IL-9R, the contribution of IL-9 signaling to neural cell-mediated pathology is still unclear (99).

Of the diseases mentioned above, there is still a need to define the exact mechanism of the role of IL-9 in diseases in which IL-9 may intensify or attenuate inflammatory responses. In the case of systemic lupus erythematosus, patients exhibited significantly higher mRNA and protein levels of IL-9 compared to healthy controls, suggesting that IL-9 is associated with promoting autoimmune inflammation (100). Yet, in a mouse model of ethanol-induced alcoholic liver injury (ALI), treatment with IL-9 can relieve the injury and reduce expression of inflammatory markers, IL-6 and TNFα, indicating that IL-9 may have an anti-inflammatory role in ALI (101). However, IL-9 may also be regulated at distinct phases of disease pathogenesis. This is evident in plasma samples from a patient population with acute or chronic stage of HIV infection. Notably, levels of IL-9 were significantly reduced with the progression of HIV-1 infection (102).

IL-9 as a therapeutic target

Blockade of IL-9 has been shown to be very effective in pre-clinical models. In murine models of intermittent allergic asthma, anti-IL-9 treatment provided strong protection against airway reactivity in a recall response (31). Anti-IL-9 is also effective in models of chronic allergen challenge (103, 104). There is also support for IL-9 being central to the pathology associated with the allergic asthmatic phenotype in patients (105). Anti-IL-9 biologics for the treatment of allergic asthma have been shown to be effective at improving asthma symptom scores in individuals with mild-to-moderate asthma; however, there were mixed results in its efficacy in the greater asthmatic population (106, 107). When MEDI-528, a humanized mAb against IL-9, was administered to patients with mild-to-moderate asthma, they observed a trend towards improved asthma symptom scores and reduced asthma exacerbation rates, while in their phase II study with patients with moderate-to-severe asthma did not experience improved clinical activities or quality of life (106,

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107). However, these studies were limited by short periods of treatment, and that allergic vs. non-allergic asthma patients were not considered separately. It is likely that asthmatic patients should be assessed for IL-9 levels in the bloodstream prior to anti-IL-9 treatment to specifically target individuals who have IL-9-driven asthmatic responses. Indeed, this may identify specific endotypes of asthma where IL-9-targeted therapies might be most effective.

Modifying IL-9 production and responses has taken a number of approaches. Retinoic acid was used to antagonize Th9-derived IL-9 and effectively reduce allergic airway inflammation (24). An IL-9R inhibitor (rhIL-9-ETA fusion toxin) effectively targets malignant cells, including Hodgkin's lymphoma, non-Hodgkin lymphoma and acute myeloid leukemia (108). IL-9Rα neutralizing antibodies were used to treat leukemia cells from several patients, demonstrated effective inhibition of leukemia cell proliferation (109). Bioniz therapeutics developed an antagonist to IL-2, IL-9, and IL-15 (BNZ132-1 now referred to as BNZ-1), while not affecting IL-4, IL-7, or IL-21 signaling (110, 111). However, inhibition of IL-9 was achieved at concentrations 10 times higher than those that resulted in inhibition of IL-2 and IL-15 (110). Whether immunotherapies involving targeting IL-9 signaling through neutralizing IL-9 or IL-9R inhibitors can be translated into the clinic requires further testing.

In contrast to blocking IL-9, adoptive cell transfer (ACT) of IL-9-producing cells has shown promise for treatment of some tumors (9, 112–115). IL-9-producing CD4+ Th9 and CD8+ Tc9 cells have shown great efficacy in pre-clinical models for cancer immunotherapies. Remarkably, tumor-specific Th9 cells present a less exhaustive and long-lived effector phenotype in comparison to antitumor effector Th1 and Th17 cells (113, 114). These studies indicate that ACT of IL-9-producing T cells can retain a strong antigen-specific antitumor effect, and this effect is clearly observed in a preclinical tumor model in which Th9 transfer was effective against melanoma (116). In melanoma patients, immune checkpoint blockade treatments targeting CTLA-4 or PD-1 using ipilimumab or nivolumab, respectively, were more effective in individuals that had elevated levels of IL-9 or IL-9-producing T cells (38, 117). Together, ACT of IL-9-producing cells presents a promising therapeutic approach for cancer treatment. Moreover, cytokines in the tumor microenvironment may also contribute to IL-9/Th9/Tc9 efficacy. Notably, IL-33 can enhance IL-9 production in murine Th9/Tc9 to enhance its antitumor activity by maintaining a central memory phenotype and upregulating cytolytic molecules, perforin and granzyme B (118). Immunotherapy with CD4+ and CD8+ chimeric antigen receptor (CAR) T cells polarized under Th9-culture conditions have enhanced antitumor activity against hematological malignancies (113, 119). This strategy is IL-9-dependent, as blockade of IL-9 after CAR Tc9 transfer attenuated anti-tumor activity (113). Nevertheless, the use of ACT/CAR T cell therapies in anticancer therapy is not without limitations, including how pre-therapy IL-9 levels and IL-9 signaling on other immune cells may impact responses to ACT/CAR T therapy. Thus, *further investigation on the safety and efficacy of this approach is needed prior to clinical application*.

IL-9 remains an attractive target in multiple pathologic conditions. Greater efforts in precision therapy will likely be necessary to identify the patients that will benefit most from agents that block IL-9 signaling or from adoptive cell therapies that provide IL-9 in the microenvironment to benefit immunity.

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Conclusions

The IL-9/IL-9R pathway has the potential to exert both pro-inflammatory and anti-inflammatory cell type-specific effects that contribute to its dual roles in disease development (Figure 1). Despite significant therapeutic advances over the last decade, the paucity of direct evidence from animal and clinical studies for the role of IL-9 in specific biological responses remains a challenge. The strongest evidence for the direct actions of IL-9 on cells highlights mast cells, innate lymphoid cells, and macrophages as mediators of disease outcomes. This is assuredly not an exhaustive list and as more detailed studies are performed, the effects of IL-9 on specific cell types and their contributions to inflammation and immunity. Better understanding of IL-9 signaling pathways and what distinguishes cell-type specific effects will provide a more complete view on how IL-9 exerts its pro-inflammatory or anti-inflammatory function in each physiological setting.

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Abbreviations

ACT	adoptive cell transfer
BMDC	bone marrow-derived dendritic cells
CAR	chimeric antigen receptor
CCR	C-C chemokine receptor
CTL	cytotoxic T lymphocyte
EAE	experimental autoimmune encephalomyelitis
IFNγ	Interferon gamma
IL	Interleukin
ILC	innate lymphoid cell
Th	T helper
TNFα	Tumor necrosis factor alpha
HDM	house dust mite
HIV	human immunodeficiency virus
HPK	Human primary keratinocyte
SLE	systemic lupus encephalitis
WT	wild type

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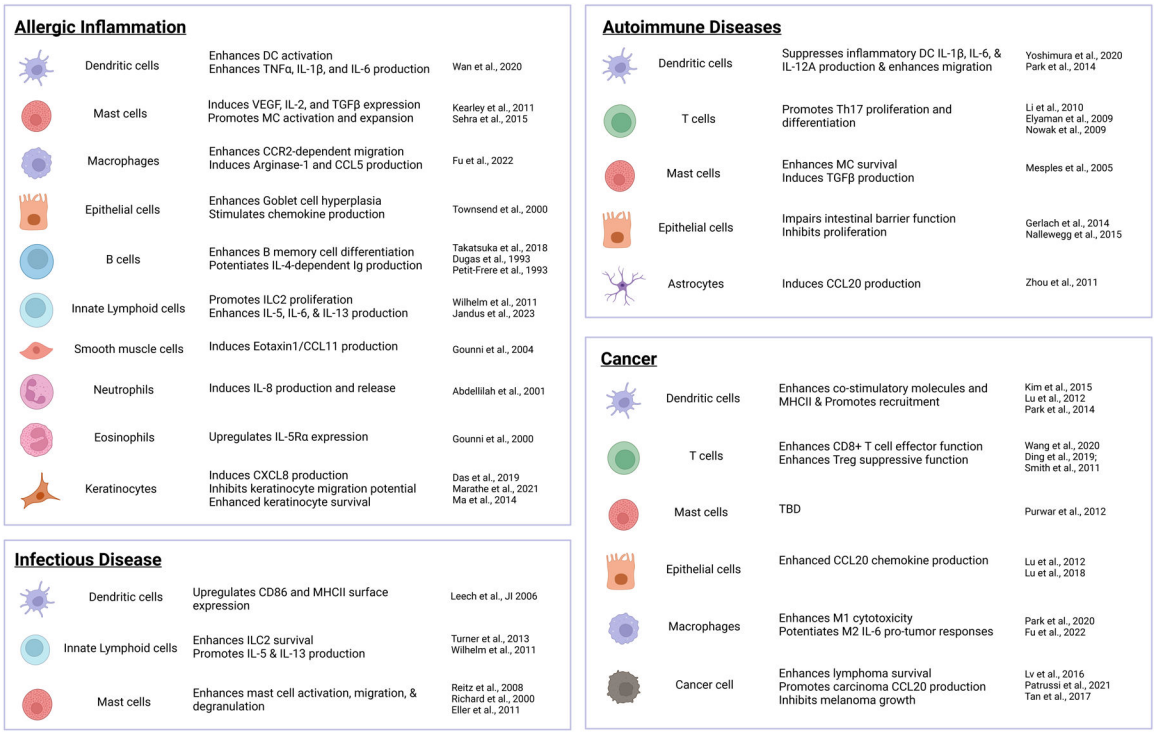

Figure 1:

Cell-type specific functions of IL-9 in inflammatory diseases. Responses of the indicated cell types to IL-9 are divided by type of immune response. Citations for each cell-type response is indicated at right.