■ REVIEW ARTICLE ■

The Role of Interleukin-9 in Asthma

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ABSTRACT
Interleukin(IL)-9 is a pleiotropic Th2 type cytokine which has been shown to play an important role in the pathogenesis of bronchial asthma. This review will concentrate on the structure, the source, and the effects of IL-9 as well as its induction and inhibition. The signaling of IL-9 through its receptor will be outlined. Moreover the role of IL-9 in airway hyperresponsiveness, mucus overproduction and airway remodeling in bronchial asthma will be discussed. Possible treatment options by blocking IL-9 will be outlined.

KEY WORDS
airway hyperresponsiveness, asthma, interleukin-9, interleukin-9 receptor, mucus overproduction

INTRODUCTION

Bronchial asthma is a complex inflammatory disorder of the airways. There are different clinical phenotypes of asthma in both adults and children. Major clinical symptoms include a variable degree of airway obstruction and airway hyperresponsiveness (AHR).[1] AHR is characterized as an exaggerated airway response to an irritant stimulus, resulting in airway obstruction in asthmatics. In asthmatic airways histopathological changes called "remodeling" can be observed. The features of this process include epithelial changes, subepithelial fibrosis, inflammatory cell infiltration, hyperplasia, and hypertrophy of the bronchial smooth muscle and mucus hypersecretion.[2,3] Airway obstruction in asthma is thought to be secondary to airway remodeling.

In asthma the airways are infiltrated by activated inflammatory cells, particularly eosinophils, T lymphocytes, and mast cells. At the site of inflammation these cells release cytokines which have been shown to play a central role in regulating inflammatory responses. Bronchial biopsies from asthmatic patients demonstrate increased expression of IL-4, IL-5, IL-9 and IL-13 when compared to control biopsies.[4-8] Other cytokines such as IL-11, IL-17 and transforming growth factor-β (TGF-β) have been associated with disease severity and airway remodeling in asthma.[9,10]

It has been suggested that IL-9 may be responsible for AHR and mucus overproduction in asthmatics.[8,11] More recently, a role for IL-9 in airway repair mechanisms leading to remodeling has been postulated.[12]

This review will aim at evidence supporting a central role for IL-9 as an important mediator in asthma. It will concentrate on the structure of IL-9, its source, its effects, its receptor (IL-9R) and both the pathological and the physiological role of IL-9 will be discussed. Moreover, the role of IL-9 in AHR, mucus hypersecretion and airway remodeling in asthma and the potential of using IL-9 "blockers" as a possible treatment in asthma will be reviewed.

STRUCTURE AND GENOMIC ARCHITECTURE OF IL-9

IL-9 belongs to the family of 4-helix bundle cytokines. This family also includes IL-2, IL-3, IL-4, IL-6, IL-7, and IL-15. Human IL-9 consists of a 14-kd glycoprotein, the mature form of which is composed of 144 amino acids along with a signal sequence of 18 amino acids. The IL-9 protein contains a high proportion of cationic amino acid residues and 10 cysteines and has 4 N-linked glycosylation sites.

The human IL-9 gene is located within the Th2 cytokine cluster region 5q31-35.[13,14] This region contains a cytokine gene cluster including IL-4, IL-5, IL-9, IL-13, and GM-CSF. These mediators have been implicated in allergic inflammation.[15,16] The IL-9 gene is composed of 5 exons and 4 introns over approximately 4 kb. The 5' flanking region shows specific binding sequences for the activator protein 1 (AP-1) and AP-2 transcription factors. A consensus sequence for IFN-regulating factor 1 was also identified in the 5' promoter region. The 5' regulatory region of the IL-9 gene has been shown to contain sequences also seen

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in similar regions of the IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, and GM-CSF genes, suggesting common regulatory mechanisms.[17] In human T cell leukemia virus type I-transformed human T cells, C5MJ2, a proximal region was found to be the minimum sequence for basal and inducible expression of the IL-9 gene.[19] Within this region a NF-κB site and its adjacent 20-base pair upstream sequence were demonstrated to play a critical role for the IL-9 promoter activity. From those studies it seems that the cooperation of different transcription factors such as NF-κB, c-Jun and others is essential for IL-9 gene expression in T cells.

SOURCES OF IL-9

A variety of cell types including T cells, mast cells, eosinophils, and neutrophils can produce IL-9. However, the major cell source of IL-9 is CD4+ T lymphocytes.[8] Stimulation of human peripheral blood mononuclear cells with anti-CD3 antibodies resulted in strong expression of IL-9 and could be further enhanced by the presence of various T cell mitogens, including phorbol myristate acetate.[19]

Mast cells being an important source of cytokines in asthmatic airways are another source of IL-9. Mast cells have been shown to produce both Th1-type and Th2-type cytokines[20-22] and they are also potent producers of IL-9.[23]

Expression of IL-9 mRNA has been detected in human peripheral blood eosinophils as well as in eosinophil-differentiated HL-60 cell lines.[24] Although the expression of IL-9 mRNA varied among individual donors there was a significant difference between the percentage of IL-9+ eosinophils in asthmatic subjects compared to normal controls.[24] Eosinophils from asthmatic subjects also release detectable levels of IL-9. Moreover, culturing these cells with either TNFα or IL-1 resulted in increased IL-9 levels in supernatants,[24] unlike eosinophils from healthy control subjects which do not spontaneously release IL-9.[24] Furthermore IL-9 mRNA positive eosinophils have been reported in bronchial biopsy specimens of asthmatics.[8]

In asthmatics neutrophils have also been found to express IL-9.[8] However, in that study the percentage rate of IL-9 mRNA expressing neutrophils was low indicating that neutrophils are not a major cellular source of IL-9.[8]

Figure 1 shows immunostaining for IL-9 protein in the submucosa of allergic airways. Immunoreactive cells stain red.

INDUCTION AND INHIBITION OF IL-9

Many cytokines are involved in IL-9 gene regulation.[19,25] IL-2 can induce IL-9 expression in human T cells. This effect can be suppressed by anti-IL-2 antibodies[19] but can be reversed by adding IL-4 and IL-10.[25] Kinetic analysis of cytokine expression suggests a cascade of cytokines that are involved in IL-9 ex-
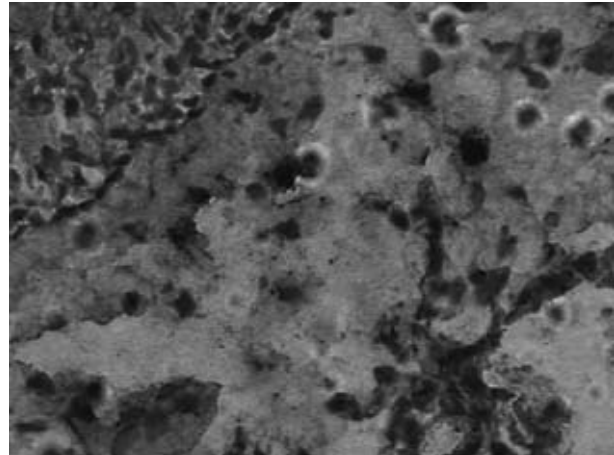

Fig. 1 Immunocytochemistry of IL-9 in allergic airway mucosa. Immunoreactive cells in the submucosa stain red. Original magnification x400.

pression. IL-2 is required for IL-4 production, a combination of IL-2 and IL-4 leads to IL-10 production, and a combination of IL-2, IL-4, and IL-10 is required for IL-9 induction.[25] IL-1, in combination either with ionomycin or IgE-antigen complexes, induced an expression of IL-9 protein and mRNA in cultured murine bone marrow-derived mast cells.[23] A marked increase in IL-9 mRNA and protein expression in these cells could also be demonstrated after stimulation with kit ligand or IL-10.[26] IL-10 as well as kit ligand increased promoter activity. IL-4R and anti-IL-10 neutralizing antibodies could reduce the production of IL-9 in murine bone marrow-derived mast cells.[23,26] Therefore regulatory mechanisms for IL-9 generation in mast cells may involve cytokines that also participate in the regulation of Th2 cells and naive CD4+ T cells. According to these findings the production of IL-9 by mast cells may play an important role in the development of late asthmatic reactions. IL-9 production can be augmented by IL-1 that can be detected in airway walls during late asthmatic reaction.[27] This effect can be further enhanced by IL-10 or kit ligand. Both mediators are produced by bronchial epithelial cells.[28,29] Intratracheal injection of kit ligand has been reported to increase airway hyperreactivity in allergic and normal mice.[30]

In atopic asthmatics increased numbers of IL-9 positive cells could be found in allergen challenged skin.[31] Segmental allergen challenge in patients with atopic asthma increased the numbers of lymphocytes expressing IL-9 as well as IL-9 protein and IL-9 mRNA in the bronchoalveolar lavage fluid.[32] These data show that IL-9 is induced via allergen challenge.

EFFECTS OF IL-9

IL-9 was originally described in the mouse as a potent T cell and mast cell growth factor.[23] IL-9 is a Th2 cy-

tokine with pleiotropic effects on various cell types. It enhances the survival of primary mast cells and induces their production of the proinflammatory cytokine IL-6.[33,34] IL-9 also stimulates the production of mast cell proteases and the expression of the high-affinity IgE receptor (FcεRIα), suggesting that IL-9 primes mast cells to respond to allergen challenge through increased cell surface expression of FcεRIα and the production of proinflammatory mediators e. g. IL-6 and proteases.[35]

IL-9 acts as a T cell growth factor in supporting antigen-independent growth of T helper cell clones.[36] IL-9 inhibits lymphokine production by IFN-γ producing CD4+ T cells and promotes proliferation of CD8+ T cells.[37] IL-9 is also able to rescue Th2 type T cell clones from apoptosis induced by deprivation of IL-2.[38] Selective overexpression of IL-9 in transgenic mice resulted in T cell infiltration of the airways *in vivo*.[39] In IL-9 transgenic mice thymic lymphomas could be observed. The tumor cells were clonal and showed double positive expression of CD 4 and CD8.[40]

Transgenic mice overexpressing IL-9 were found to have increased serum levels of all Ig isotypes including IgE and an expansion of lymphocytes from the B1 subset (Mac+ IgM+) could be observed.[41,42] Furthermore, accumulation of B cells was detected in the lungs of IL-9 transgenic mice. IL-9 has been shown to enhance IL-4 mediated IgE production in both human and murine B cells.[43]

Marked eosinophilia has been demonstrated in transgenic mice overexpressing IL-9 and in naive mice after intratracheal administration of recombinant IL-9.[39,44] Recently, IL-9 was found to promote eosinophil maturation in synergy with IL-5.[45] IL-9 enhances the expression of IL-5Rα on human eosinophils.[46] In that study, IL-9 inhibited eosinophil apoptosis in a dose-dependent manner. Moreover, IL-9 enhanced eosinophil development. IL-9 might therefore induce airway eosinophilia by upregulating the IL-5 response and potentiating the IL-5-mediated maturation of eosinophil precursors.[46] Allergen-induced elevated expression of IL-9 in the skin and the lungs of atopic asthmatics was correlated with increased numbers of eosinophils.[31,32] These findings further underline the role of IL-9 as an important factor in inducing eosinophilia in allergic inflammation.

Expression of IL-9Rα has been demonstrated in freshly isolated human peripheral blood polymorphonuclear cells (PMN) as well as in BAL-derived PMN from asthmatic patients.[47] IL-9 induces the production and release of IL-8 by human neutrophils from asthmatic subjects. This effect could be blocked by anti-IL-9 neutralizing antibody.[47]

Overexpression of IL-9 in transgenic mice resulted in increased expression of eotaxin, macrophage inflammatory protein 1α, and monocyte chemotactic proteins 1, 3, and 5 in lung epithelial cells.[44] Elevated chemokine expression could also be demonstrated in cultured primary epithelial cells and in epithelial cell lines stimulated with IL-9 even in the absence of IL-4.[44] IL-9 was found to stimulate the release of the T cell chemotactic factors IL-16 and regulated on activation, normal T cells expressed and secreted (RANTES) from human bronchial epithelial cells.[48] Furthermore, IL-9 has been shown to stimulate mucin transcription and upregulate mucus expression in airway epithelial cells.[11,49]

Recently it has been demonstrated that IL-9 increased TNFα induced IL-8 release and IL-13 induced eotaxin release from airway smooth muscle cells.[50] This effect was shown to be mediated via phosphorylation of ERK.[50]

According to these data IL-9 has pleiotropic activities on various cell types that play an important role in the pathogenesis of asthma. Unlike other cytokines such as IL-4 or IL-5, IL-9 seems to primarily enhance the activities of other cytokines and factors. This could be important in terms of antagonizing IL-9 activity in the way that inhibiting IL-9 may less likely compromise normal host defense than the blockage of other cytokines. Figure 2 summarizes the effects of IL-9.

IL-9 RECEPTOR

The IL-9 receptor (IL-9R) belongs to the hematopoietin receptor superfamily which includes many other cytokine receptors e. g. IL-2, IL-3, IL-4, IL-5, IL-7, and IL-10 receptor. The IL-9R is formed by a ligand specific α subunit (IL-9Rα) and a common γc chain that is also found in IL-2, IL-4, IL-7, and IL-15 receptor complexes.[51] The human IL-9RA gene is composed of 11 exons and 10 introns ranging approximately over 17 kb.[42] Analysis of the 5' flanking region revealed no TATA box. Possible binding sequences for AP-1, AP-2, AP-3, and nuclear factor κB was demonstrated[52] illustrating a potential transcriptional control of the IL-9RA gene.

The signal transduction of IL-9 is thought to be mediated through the Janus kinase-signal transducer and activator of transcription (STAT) pathway. Constitutive association of Janus kinase 1 with IL-9R was observed.[53] IL-9-induced activated complexes containing STAT-1, STAT-3, and STAT-5 were also found. This finding was confirmed by the observed presence of STAT-1 and STAT-3 homodimers and STAT-1 / STAT-3 heterodimers after heteromerization of IL-9Rα and common γc subunits.[54] Activation of STAT factors is required in IL-9-induced proliferation and inhibition of glucocorticoid-induced apoptosis in T cells.[54,55] Recently IL-9 was found to induce transient phosphorylation of MEK, ERK2 and p90/RSK in murine lymphoid and mast cell lines.[56] That study indicated that IL-9 could transiently activate the mitogen-activated protein kinase pathway and thus contribute to growth stimulation of hematopoetic cell
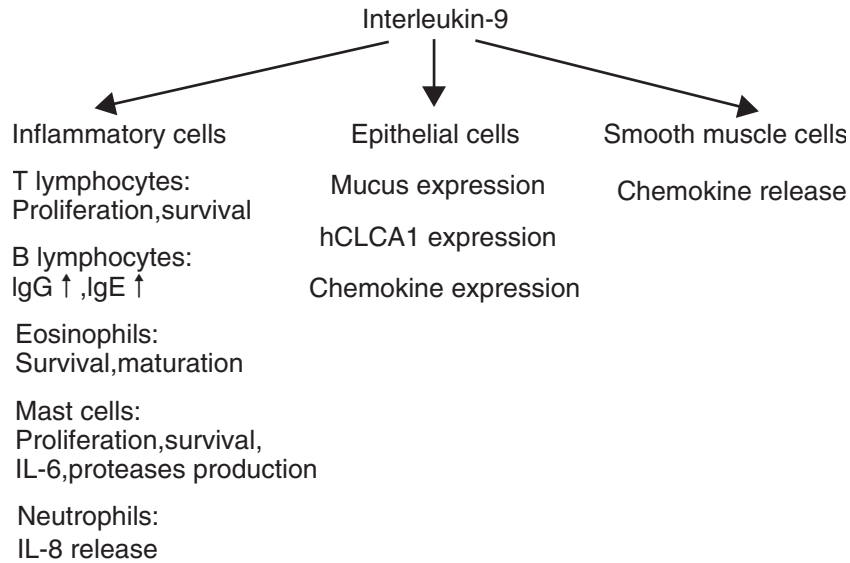
Interleukin-9

Inflammatory cells

T lymphocytes:
Proliferation, survival

B lymphocytes:
IgG ↑, IgE ↑

Eosinophils:
Survival, maturation

Mast cells:
Proliferation, survival,
IL-6, proteases production

Neutrophils:
IL-8 release

Epithelial cells

Mucus expression

hCLCA1 expression

Chemokine expression

Smooth muscle cells

Chemokine release

Fig. 2 Effects of IL-9 on inflammatory cells, epithelial cells, and smooth muscle cells.
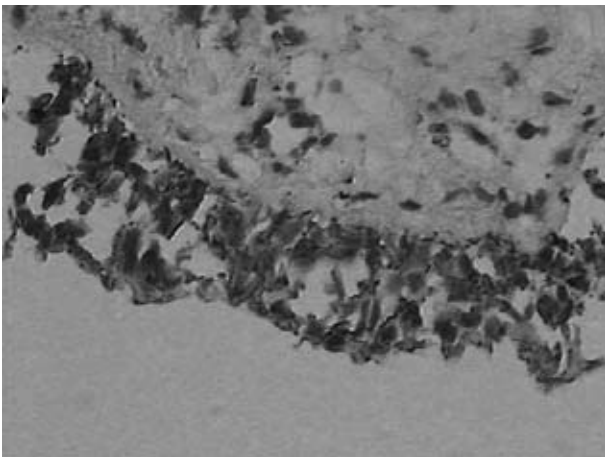

Fig. 3 Immunocytochemistry of IL-9R in allergic airway mucosa. Immunoreactive cells in the epithelium stain red. Original magnification x400.

lines.[56]

Figure 3 shows immunostaining of IL-9R protein in the epithelium of allergic airway mucosa. Almost all the epithelial cells express IL-9R (red cells).

IL-9 AND AIRWAY HYPERRESPONSIVE-NESS

Airway hyperresponsiveness (AHR) is one of the hallmark symptoms of bronchial asthma.[1] The chromosomal region 5q31-33 has been identified to contain candidate genes for AHR.[15] This region contains several gene candidates for cytokines, growth factors, and growth factor receptors that may play an important role in asthmatic inflammation.[16] The IL-9 gene has

been localized to this region.[13,14]

The *in vivo* importance of IL-9 in the pathophysiology of asthma was first determined in animal models of airway inflammation. Nicolaides *et al.* have shown a close association between the IL-9 gene and bronchial hyperresponsiveness.[14] In that study analysis of the murine IL-9 gene identified a genetic defect at the C57BL/6(B6) IL-9 locus associated with a lack of IL-9 expression in the lung and reduced AHR in naive B6 mice. In that same study an increased expression of IL-9 gene and protein in the lung was associated with AHR in DBA/J2 mice. The tight genotype-phenotype relation was further supported by the finding that (B6D2)F$_1$ mice were found to be intermediate in both lung IL-9 levels and airway responsiveness.[14]

Selective overexpression of the IL-9 gene in the airways of transgenic mice resulted in massive airway inflammation, with infiltration of eosinophils and lymphocytes, mast cell hyperplasia, and increased subepithelial collagen deposition.[39,44] Moreover, elevated IgE levels, AHR, and increased responsiveness to antigen stimulation could be observed. In contrast, overexpression of IL-4 using the CC-10 promoter resulted in baseline eosinophilia without AHR.[57]

Instillation of recombinant mouse IL-9 into the airways of B6 mice for up to 10 days resulted in time-dependent and dose-dependent increases in AHR, BAL eosinophilia, elevated IgE levels, increased lung proteases, and submucosal membrane thickening.[58]

Shimbara *et al.* investigated the contribution of IL-9 to the pathogenesis of asthma.[8] In that study IL-9 and IL-9R protein and mRNA expression in bronchial tissue from subjects with atopic asthma, sarcoidosis, and from atopic and nonatopic healthy control subjects were compared. IL-9 was highly expressed in

asthmatic airways in comparison to the other disease groups. In respect to cell type, T cells and, to a lesser extent, eosinophils and neutrophils were the main cellular sources of IL-9 production. Furthermore, both the degree of airflow obstruction (FEV1) and airway responsiveness to metacholine were significantly correlated with IL-9 mRNA expression.[8] Lamblin *et al.* reported that patients with nasal polyposis (NP) associated with asymptomatic AHR have an eosinophilic bronchial inflammation similar to that of asthmatic patients with NP.[59] To better evaluate the role of IL-9 in AHR, bronchial specimens from patients with NP alone, NP associated with AHR, and NP associated with asthma were examined. An increased number of IL-9 mRNA+ cells were detected in subjects with asthma and AHR in comparison to subjects with NP alone. However, no significant difference could be demonstrated between subjects with asthma and subjects with AHR. These data further support the role of IL-9 in mediating AHR.

IL-9 AND MUCUS HYPERSECRETION

Mucus overproduction is frequently observed in asthmatics. Several reports have demonstrated upregulation of mucus production by IL-9.[11,49] In transgenic mice constitutive IL-9 expression resulted in elevated MUC2 and MUC5A/C gene expression in airway epithelial cells and PAS-positive staining. Intratracheal instillation of IL-9 led to similar findings in C57BL/6J mice.[11] IL-9 also induced expression MUC2 and MUC5A/C in human primary lung cultures and in the human mucoepidermoid NCI-H292 cell line *in vitro*.[11] These data indicate that IL-9 upregulates mucus production through induction of mucin gene expression.

Recently, the mechanism by which IL-9 leads to mucus over-production has been further elucidated. Expression of the calcium-activated chloride channel mCLCA3 in the lungs of a murine model of allergic asthma was associated with AHR and mucus overproduction.[60] Furthermore, introduction of mCLCA3 or its human counterpart hCLCA1 into the human mucoepidermoid cell line NCI-H292 induced mucus production as well as MUC5A/C expression.[60] In IL-9 transgenic mice induction of mCLCA3 has been identified in the lung epithelium.[61] In that study, expression of mCLCA3 was induced in the lungs of antigen-exposed mice, and this induction could be suppressed by treatment with IL-9 neutralizing antibodies. The chloride channel hCLCA1 could be induced *in vitro* in human primary lung cells by Th2 cytokine treatment.[61] Our group found an upregulation of hCLCA1 in mucus producing epithelium of asthmatics compared to control subjects.[62] A strong correlation between hCLCA1 mRNA and IL-9 as well as IL-9 R positive cells could be observed. This finding indicates that IL-9 may act through hCLCA1 and that this channel may be in part responsible for mucus over-

production in bronchial asthmatics.[62]

IL-9 AND AIRWAY REMODELING

Recent studies have suggested that IL-9 may play an important role in airway remodeling in asthma. Increased expression of IL-9 supports eosinophil infiltration and survival.[45,46] IL-9 induces the release of T cell chemoattractant factors from bronchial epithelial cells[48] thus contributing to inflammtion. Further IL-9 has also been shown to induce mucus expression.[11,49] In a recent study IL-9 was reported to have little effect on mucus production in fully differentiated ciliated human airway epithelia.[12] In contrast in the setting of airway injury repair IL-9 induced goblet cell hyperplasia. This effect could also be observed when epithelium was exposed to IL-9 before it was fully differentiated.[12] These findings suggest that increased IL-9 expression during airway epithelial repair may generate elevated numbers of goblet cells in asthmatics. Experiments in our own laboratory using the cell lines showed induction of collagen 1 by stimulation with IL-9 (unpublished observations).

NEUTRALIZING IL-9 ACTIVITY AS A POTENTIAL TREATMENT

Neutralizing antibodies as well as soluble receptors have demonstrated involvement of IL-4, IL-5 and IL-13 in asthmatic responses.[63-65] These strategies may also offer an opportunity to inhibit selectively asthmatic inflammation without major side effects as seen with the use of glucocorticosteroids. However, by inhibiting these cytokines not only asthmatic inflammation is decreased but other important regulatory functions of these cytokines are also abolished, thus affecting the normal host defense system.

Two studies using IL-9 neutralizing antibodies demonstrated that IL-9 is an important mediator of asthma.[66,67] In one model, exposure of (B6D2) F1 to either *A. fumigatus* or dust mite antigen resulted in a marked allergic inflammatory response including significant increases in BAL eosinophils, elevated serum total IgE levels, increased mucin production, and AHR in comparison to control or naive animals. Intratracheal administration of IL-9 neutralizing antibodies reduced AHR, serum total IgE elevations, and led to increases in BAL eosinophils compared to treatment with isotype-specific control antibody or untreated sensitized mice. Alcian Blue/PAS stains for mucin as well as overall histopathologic grading confirmed that the antibody treatment effectively abrogated the allergic response in antigen-challenged animals.[66] In the other study systemic administration of IL-9 neutralizing attenuated pulmonary eosinophilia and lung damage, the goblet cell hyperplasia, and AHR induced by antigen challenge with ovalbumin but had no effect on IL-4, IL-5, and IL-13 mRNA levels in the lungs.[67] In that study (B6D2) F1 (moderately hyperresponsive to bronchoconstrictor challenge) and C57B16J (hypore-

sponsive to bronchoconstrictor agents) mice were studied. Interestingly in a mouse model immunization with antiIL-9 antibody abrogated mast cell protease-1 levels as well as eosinophilia after implantation of an IL-9 secreting tumor.[68] Moreover, in that same model antiIL-9 antibody immunization inhibited *Trichuris muris* -induced blood eosinophilia.[68] However, vaccinated mice were not able to expel the worm. The first IL-9 deficient mice were reported as being healthy. No abnormalities in development or phenotype could be observed.[69] Naive cytokine profiles, Th1 and Th2 cell development, and generation of naive or antigen-specific antibody responses were normal.[69]

Taken together neutralizing IL-9 may offer a promising approach to the blocking of asthmatic responses. However, attenuation of IL-9 could possibly compromise defense against parasites.

ACKNOWLEDGEMENTS

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