

Interleukin-4 and Interleukin-5 as Targets for the Inhibition of Eosinophilic Inflammation and Allergic Airways Hyperreactivity

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Clinical and experimental investigations suggest that allergen-specific CD4⁺ T-cells, IgE and the cytokines IL-4 and IL-5 play central roles in initiating and sustaining an asthmatic response by regulating the recruitment and/or activation of airways mast cells and eosinophils.

IL-5 plays a unique role in eosinophil development and activation and has been strongly implicated in the aetiology of asthma. The present paper summarizes our recent investigations on the role of these cytokines using cytokine knockout mice and a mouse aeroallergen model. Investigations in IL-5^{-/-} mice indicate that this cytokine is critical for regulating aeroallergen-induced eosinophilia, the onset of lung damage and airways hyperreactivity during allergic airways inflammation. While IL-4 and allergen-specific IgE play important roles in the regulation of allergic disease, recent investigations in IL-4^{-/-} mice suggest that allergic airways inflammation can occur via pathways which operate independently of these molecules. Activation of these IL-4 independent pathways are also intimately associated with CD4⁺ T-cells, IL-5 signal transduction and eosinophilic inflammation. Such IL-5 regulated pathways may also play a substantive role in the aetiology of asthma. Thus, evidence is now emerging that allergic airways disease is regulated by humoral and cell mediated processes. The central role of IL-5 in both components of allergic disease highlights the requirements for highly specific therapeutic agents which inhibit the production or action of this cytokine.

Key words: asthma - interleukin-4 - interleukin-5 - eosinophils

Characterization of respiratory secretions and biopsy samples from asthmatics suggests that inflammatory cells play a central role in the clinical expression and pathogenesis of the disease (Gleich & Adolphson 1986, Djukanovic et al. 1990, Bochner et al. 1994). Clinical investigations show a correlation between the presence of activated inflammatory cells, morphological changes to airways tissue and the development of airways hyperreactivity (De Monchy et al. 1985, Fukuda et al. 1985, Gleich et al. 1988, Wardlaw et al. 1988, Beasley et al. 1989, Frick et al. 1989, Motojima et al. 1989, Bousquet et al. 1990, Broide et al. 1991, Walker et al. 1991a,b, Ohashi et al. 1992). The cellular composition of the inflammatory infiltrate in the airways is complex, consisting of increased numbers of activated eosinophils, mast cells, monocytes and neutrophils and the recruitment and/or activation of these cells appears to be controlled by the secretion of cytokines and chemotactic

agents from antigen-stimulated T-lymphocytes (T cells) (Azzawi et al. 1990, Bradley et al. 1991, Ohashi et al. 1992, Walker et al. 1992a,b). While activated leukocytes and the mediators they release contribute to a complex inflammatory cascade, the precise role that individual inflammatory cells and mediators play in the events which initiate the morphological and functional changes of the asthmatic lung are unknown.

Recently, there has been increasing interest in the involvement of CD4⁺ T-cells and eosinophils and the molecules which regulate the effector function of these cells, in the pathophysiology of asthma. There is accumulating evidence from clinical investigations and animal models of allergic airways inflammation that CD4⁺ Th₂ type lymphocytes and eosinophils play a critical role in both the induction and pathogenesis of asthma (Bradley et al. 1991, Walker et al. 1992a,b, Foster et al. 1996). A number of investigations have shown a correlation between the accumulation of activated CD4⁺ T-cells and eosinophils, their inflammatory products in the lung and disease severity (De Monchy et al. 1985, Fukuda et al. 1985, Gleich et al. 1988, Wardlaw et al. 1988, Beasley et al. 1989, Frick et al. 1989, Azzawi et al. 1990, Bousquet et

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al. 1990, Broide et al. 1991, Walker et al. 1991a,b, Bentley et al. 1992, Ohashi et al. 1992).

While the chain of events leading to asthma involves a complex cascade of interacting cells and inflammatory mediators, it is becoming apparent that the cytokines interleukin (IL)-4 (IL-4) and IL-5 secreted from allergen-specific CD4⁺ Th₂ type cells play central roles in initiating and sustaining an asthmatic response by regulating the recruitment and/or activation of airways mast cells and eosinophils (Wierenga et al. 1990, Walker et al. 1991a,b, Robinson et al. 1992). Increased levels of IL-4 and IL-5 are found in the respiratory secretions from atopic asthmatics (Walker et al. 1991a,b). Furthermore, an increase in the number of BALF cells from atopic asthmatics that express elevated levels of mRNA for IL-4 and IL-5 correlates with the level of bronchial responsiveness to spasmogenic stimuli (Robinson et al. 1993a,b).

IL-4 is a critical factor for the regulation of T-cell commitment to the CD4⁺ Th₂ phenotype and plays an essential role in IgE isotype switching in B cells (Snapper & Paul 1987, Finkelman et al. 1988, Berstedt-Lindqvist et al. 1988, Swain et al. 1990, Kopf et al. 1993, Dubucquoi et al. 1994). By contrast, IL-5 regulates the growth, differentiation and activation of eosinophils (Campbell et al. 1987, Lopez et al. 1988, Yamaguchi et al. 1988) and provides an essential signal for the recruitment of this leukocyte to the lung during allergic inflammation (Coffman et al. 1989, Foster et al. 1996). These cytokines may also regulate eosinophil trafficking by activating adhesion-systems at the vascular endothelium (Walsh et al. 1990; Schleimer et al. 1992). IgE and allergen-specific immunoglobulins produced after isotype switching may play key roles in mast cell activation and potentially eosinophil degranulation (Looney et al. 1986, Gounni et al. 1994, Dubucquoi et al. 1994, Kaneko et al. 1995). Recently, IgE dependent mechanisms have also been suggested to play an important role in Th₂ cell cytokine production and in the development of airways eosinophilia and eosinophil associated airways dysfunction (Eum et al. 1996, Coyle et al. 1996).

The key roles of IL-4 and IL-5 in the development and maintenance of allergic disease has identified these molecules as important targets for pharmacological modification of the inflammatory response associated with asthma (Anderson & Coyle 1994). Furthermore, both cytokines have been implicated in the development of airways hyperreactivity to spasmogens after antigen inhalation (Mauser et al. 1993, Iwama et al. 1993, Eum et al. 1995, Corry et al. 1996, Foster et al. 1996). However, in a number of investigations with asthmatics and animal models of asthma, a correlation be-

tween increased eosinophil numbers and the development of airways hyperreactivity has not been observed (Djukanovic et al. 1990, Elwood et al. 1992, Eum et al. 1995, Corry et al. 1996). Furthermore, the comparative importance of IL-4 and IL-5 to the induction of aeroallergen-induced airways hyperreactivity is controversial (Gulbenkian et al. 1992, Iwama et al. 1993, Mauser et al. 1993, Nagai et al. 1993, Van Oosterhout, et al. 1993, Yamaguchi et al. 1994, Foster et al. 1996, Corry et al. 1996).

Recently, mouse models which mimic certain phenotypic characteristics of late-phase asthmatic responses have been used to investigate the pathophysiology of allergic airways disease. Late-phase reactions are considered to reflect more closely the clinical expression of asthma and, therefore, serve as an important model for experimental investigations. Ovalbumin sensitisation by systemic injection followed by aeroallergen challenge induces pronounced allergic airways disease which is dependent on CD4⁺ Th₂ type cells. Aeroallergen-induced allergic disease is characterised by asthmalike pathological changes to the airways, enhanced bronchial reactivity to spasmogenic stimuli, airways eosinophilia and the production of OVA-specific IgE and IgG₁. Furthermore, the Th₂ cytokines IL-4 and IL-5 have been identified as the central mediators in the development of the pathophysiological features of the allergic response.

Recently, we have used a mouse model of allergic airways inflammation and IL-4 deficient (IL-4^{-/-}) mice and IL-5^{-/-} mice to define the roles of these cytokines in the development of aeroallergen-induced lung damage and airways hyperreactivity. In mice made deficient in IL-5 production by the targeted disruption of the IL-5 gene in embryonic stem cells, no obligatory role for this cytokine was demonstrated in the regulation of conventional B cells, in normal T cell dependent antibody responses or in cytotoxic T cell development (Kopf et al. 1996, see also Matthaei et al. this issue). IL-5^{-/-} mice are specifically affected in IL-5 production with no evidence of secondary effects on other cytokines. IL-4^{-/-} mice do not produce IgE and have impaired CD4⁺ Th₂ cell responses (Kopf et al. 1993).

ROLE OF IL-5 IN ALLERGIC AIRWAYS DISEASE

Characterization of allergic responses in the airways of IL-5^{-/-} mice indicated an essential role for this cytokine in the development of allergic airways disease (Foster et al. 1996). In IL-5^{-/-} mice, the airways hyperreactivity, eosinophilia and gross changes in airway structure normally resulting from aeroallergen challenge was abolished. In contrast with eosinophils, neutrophil and lymphocyte numbers in the BALF of IL-5^{-/-} mice were still signifi-

cantly elevated following aeroallergen challenge. However, accumulation of lymphocytes was less marked in IL-5^{-/-} mice than in control animals, which may reflect the diminished inflammatory response to aeroallergen exposure and the role of IL-5 in enhancing T-cell adhesion to vascular endothelial cells (Walsh et al. 1990). OVA-specific IgE was detected at similar levels in the sera from wild type and IL-5^{-/-} mice after aeroallergen exposure, indicating that IgE and eosinophilia are independently regulated, as previously shown in pulmonary parasitic infestation (Coffman et al. 1989).

The central role of IL-5 in aeroallergen-induced eosinophilic recruitment into the airways was confirmed by reconstituting IL-5 production in the lungs of IL-5^{-/-} mice using recombinant vaccinia viruses (rVV), engineered to express this cytokine (rVV-HA-IL-5). In the lungs of IL-5^{-/-} mice exposed to rVV-HA-IL-5 and OVA, severe pulmonary eosinophilic inflammation, characteristic lung pathology and airways hyperreactivity were completely restored. Airways disease was also established by adoptively transferring CD4⁺ Th₂ type cells from sensitised and aeroallergen-challenged wild type mice to IL-5^{-/-} mice which were naive, but aerosolised with OVA. These investigations established that IL-5 and CD4⁺ Th₂ type cells play a pivotal role in generating blood and airways eosinophilia and in the subsequent development of airways hyperreactivity and lung damage, that occurs in response to aeroallergens. These investigations also implicated eosinophils as the primary pro-inflammatory cell involved in the induction of changes in pulmonary structure and function.

Notably, the lungs of IL-5^{-/-} mice given VV-HA-IL-5 in the absence of OVA sensitisation and aerosolisation, showed little evidence of eosinophil recruitment (2-fold increase over basal levels) and accumulation was not accompanied by dense cellular infiltration or in changes in lung morphology or function. It was verified that IL-5 was expressed in the lungs of mice at concentrations which induced physiological responses. These results, therefore, suggest that IL-5 alone is not directly responsible for enhanced bronchial responsiveness to spasmogens (Van Oosterhout et al. 1993). Furthermore, these results indicate that IL-5 is a poor chemotactic agent for eosinophils in the lung and that other factors derived from the site of antigen presentation are involved in the amplification of the signal for eosinophil migration, and are essential for widespread eosinophilic inflammation and degranulation and the onset of respiratory disease.

Recently, adoptive transfer of OVA-primed CD4⁺ Th₂ cells from sensitised wild type mice, to sensitised and non sensitised IL-5^{-/-} mice, was

shown to reconstitute aeroallergen-induced lung damage and airways hyperreactivity (Hogan et al. unpublished observation) These adoptive transfer experiments demonstrated that changes in lung resistance and airways eosinophilia are directly associated with the presence of antigen activated CD4⁺ T cells in the airways and that allergic responses can be cell-mediated and occur in the absence of humoral immunity.

ROLE OF IL-4 IN ALLERGIC AIRWAYS DISEASE

Recently, the role of IL-4 in the onset of allergic airways inflammation was investigated in IL-4^{-/-} mice (Brusselle et al. 1994). The lungs of sensitised wild type mice exposed to aerosolised antigen (OVA) showed highly localized pockets of airways inflammation, which were primarily characterized by eosinophilic infiltration. In comparison to lung eosinophil numbers, neutrophil and lymphocyte levels were only slightly elevated, and macrophage numbers did not increase, following aeroallergen challenge. In this model of allergic inflammation, aeroallergen-challenge did not induce pathological changes to airways tissues and changes in airways reactivity to spasmogens was not established. Notably eosinophil accumulation, but not neutrophil or lymphocyte infiltration, was significantly reduced in aeroallergen challenged IL-4^{-/-} mice in comparison to wild type mice (Brusselle et al. 1994).

In a similar series of experiments in this laboratory using a slightly different protocol for the induction of allergic airways inflammation (Hogan et al. 1997a) and IL-4^{-/-} mice, we have also shown that the recruitment of eosinophils to the lung, but not to the blood, was impaired (but not abolished) in IL-4^{-/-} mice derived from the same lineage (Hogan et al. 1997a). However, the characteristic lung damage and airways hyperreactivity to β -methacholine, induced by aeroallergen challenge, was not attenuated. These investigations indicate that airways eosinophilia, extensive lung damage and airways hyperreactivity can occur in the absence of IL-4, antigen-specific production of IgE and in the presence of impaired CD4⁺ Th₂ cell responses. The characteristic lung damage and airways hyperreactivity induced by aeroallergen challenge, was only inhibited in IL-4^{-/-} mice after pre-treatment with anti-IL-5 mAb or anti-CD4⁺ mAb and the subsequent abolition of blood and airways eosinophilia. Furthermore, when activated through the CD3-TCR complex CD4⁺ T cells isolated from sensitised aeroallergen challenged IL-4^{-/-} mice produced significant amounts of IL-5. These results confirm observations in IL-5^{-/-} mice implicating this cytokine and eosinophilic inflammation as central mediators in the pathogenesis of allergic

lung disease. Furthermore, our results suggest that IL-4 is not required for the development of all CD4⁺ T-cells which regulate eosinophilia and the induction of pathological changes in pulmonary tissue and airways hyperreactivity during allergic disease. Eosinophilic inflammation has also been observed in models of parasite infestation and malaria when IL-4 was absent during the initiation of the immune response (Coffman et al. 1989, Von der Weid et al. 1994). Thus, evidence is accumulating that CD4⁺ T-cell subsets exist which do not require IL-4 for dedication into a phenotype that produces IL-5 and regulates eosinophilia. The pathway that activates aeroallergen-induced pulmonary eosinophilic inflammation in an IL-4 independent manner may function to supplement responses by classical CD4⁺ TH₂-type cells.

There is increasing interest in the expression of IL-4 and IL-5 by T cells in various diseases and the lineages of these cells (Swain 1994, Sewell & Mu 1996). Interestingly, intrinsic asthmatics show no correlation between disease and IgE production. Furthermore, respiratory secretions from these individuals are characterized by increased levels of IL-5 (but not IL-4) and activated CD4⁺ T-cells (Walker et al. 1992a,b, Bochner et al. 1994). Characterization of the phenotype of CD4⁺ T-cells producing IL-5 in IL-4^{-/-} mice and the factors involved in their selection and activation may provide important insights into the aetiology of intrinsic asthma. It will be of particular interest to determine if these CD4⁺ T cells can also produce IL-4 and provide help for IgE production.

These observations in IL-4^{-/-} mice have significant implications for the development of strategies for the treatment of airways dysfunction in asthma. Therapeutic approaches which focus solely on inhibiting the action of IL-4 and allergen-specific IgE may not be effective in relieving airways obstruction associated with allergen provocation.

These findings are also discordant to the suggestion that activation of IL-4 dependent pathways are critical for the regulation of allergic airways diseases and that IL-5 and eosinophils are not required. Corry et al. (1996) recently reported that pretreatment of BALB/c mice with anti-IL-4 mAb during the period of systemic sensitisation, but not during aeroallergen challenge, significantly reduced airways hyperreactivity and airways eosinophilia. However, administration of anti-IL-5 mAb inhibited airways eosinophilia, but did not attenuate airways hyperreactivity. The innate contribution of IL-4, IL-5 and the effector pathways of eosinophils and mast cells, in conjunction with different sensitisation protocols, to the induction of allergic airways hyperreactivity in C57BL/6 in comparison to BALB/c mice, was postulated to

explain these discordant observations (Drazen et al. 1996). According to this hypothesis, C57BL/6 mice are genetically deficient in mast cell derived inflammatory mediators, which may predispose this strain to resistance of mast cell-mediated airways hyperreactivity. In contrast to C57BL/6 mice, BALB/c mice produce high levels of IL-4 and IgE in response to sensitisation. Thus, airways hyperreactivity was proposed to be predominantly regulated by IgE-mediated activation of mast cells in BALB/c mice and solely regulated by an IL-5-eosinophil dependent mechanism in C57BL/6 mice. The relative contribution of these two mechanisms to the induction of allergic airways dysfunction in various strains of mice was suggested to account for the differences observed in these studies (Drazen et al. 1996). However, investigations on the role of IL-4 in allergic airways dysfunction not only differ in the strain of mice used but also in the route of sensitisation [i.p. (Foster et al. 1996) vs subcutaneous (Corry et al. 1996)] and in aeroallergen challenge protocols. Thus, in murine models of allergic inflammation, the mode of sensitisation and the severity of aeroallergen challenge may regulate the degree of the airways inflammation and the requirement for various factors during antigen processing for the subsequent onset of disease in response to recall antigens. In any event, aeroallergen-induced airways disease can occur independently of IL-4.

EVIDENCE FOR MULTIPLE PATHWAYS FOR THE REGULATION OF PULMONARY EOSINOPHILIA AND AIRWAYS DYSFUNCTION

Experiments in IL-4^{-/-} and IL-5^{-/-} mice suggest that aeroallergen induced airways eosinophilic inflammation, lung damage and airways hyperreactivity can be regulated by at least two pathways which centre on the activation of IL-5 signal transduction processes. Investigations in these factor-deficient mice also support the concept that allergic airways disease is not only induced by humoral but also cell mediated immunological mechanisms. The proposal that multiple pathways may regulate allergic airways hyperreactivity has important implications for the treatment of human asthma and in understanding the pathophysiology of this disorder. Furthermore, if eosinophil -dependent and -independent pathways contribute to the pathogenesis of allergic disease this would clarify the increasing number of discordant observations on the role of this leukocyte in the induction of aeroallergen-induced airways hyperreactivity in animal models. Recently, in BALB/c mice a role for IL-5 and eosinophils in the initiation of bronchial hyperreactivity and morphological changes to the airways during allergic airways inflamma-

tion was established (Hogan et al. 1997b). Sensitisation and repetitive aerosolisation of mice with ovalbumin resulted in a severe airways inflammatory response which directly correlated with the induction of extensive airways damage and bronchial hyperreactivity to β -methacholine. Treatment of mice with anti-IL-5-mAb before aeroallergen-challenge, abolished blood and airways eosinophilia, and lung damage and significantly reduced (but did not abolish) bronchial hyperreactivity. These results show that IL-5 and associated eosinophilia contribute to the development of aeroallergen-induced bronchial hyperreactivity and are essential for the development of morphological changes in the airways. Moreover, data indicates that there are at least two distinct pathophysiological mechanisms for the development of allergen-induced bronchial hyperreactivity, and that the induction of allergic airways disease is not exclusively regulated by IL-4.

The essential and specific role of IL-5 in regulating blood and tissue eosinophilia, and the subsequent involvement of this leukocyte in the induction of lung damage and airways dysfunction identifies IL-5 as a primary therapeutic target for the relief of airways dysfunction in asthma. Developing strategies to inhibit IL-5 production and action, such as by the delivery of inhibitory cytokines to the lung, which specifically down regulate IL-5 producing T cell responses and eosinophilic inflammation, may be more beneficial than the current drugs of choice for preventative treatment and in the relief of acute and chronic asthma and allergic disease.

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