

## IL-5 and IL-5 Receptor in Asthma

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*Eosinophils, along with mast cells are key cells involved in the innate immune response against parasitic infection whereas the adaptive immune response is largely dependent on lymphocytes. In chronic parasitic disease and in chronic allergic disease, IL-5 is predominantly a T cell derived cytokine which is particularly important for the terminal differentiation, activation and survival of committed eosinophil precursors. The human IL-5 gene is located on chromosome 5 in a gene cluster that contains the evolutionary related IL-4 family of cytokine genes. The human IL-5 receptor complex is a heterodimer consisting of a unique  $\alpha$  subunit (predominantly expressed on eosinophils) and a  $\beta$  subunit which is shared between the receptors for IL-3 & GM-CSF (more widely expressed). The  $\alpha$  subunit is required for ligand-specific binding whereas association with the  $\beta$  subunit results in increased binding affinity. The alternative splicing of the  $\alpha$ IL-5R gene which contains 14 exons can yield several  $\alpha$ IL-5R isoforms including a membrane-anchored isoform ( $\alpha$ IL-5Rm) and a soluble isoform ( $\alpha$ IL-5Rs). Cytokines such as IL-5 produce specific and non-specific cellular responses through specific cell membrane receptor mediated activation of intracellular signal transduction pathways which, to a large part, regulate gene expression. The major intracellular signal transduction mechanism is activation of non-receptor associated tyrosine kinases including JAK and MAP kinases which can then transduce signals via a novel family of transcriptional factors named signal transducers and activators of transcription (STATS). JAK2, STAT1 and STAT 5 appear to be particularly important in IL-5 mediated eosinophil responses.*

*Asthma is characterized by episodic airways obstruction, increased bronchial responsiveness, and airway inflammation. Several studies have shown an association between the number of activated T cells and eosinophils in the airways and abnormalities in FEV1, airway reactivity and clinical severity in asthma. It has now been well documented that IL-5 is highly expressed in the bronchial mucosa of atopic and intrinsic asthmatics and that the increased IL-5 mRNA present in airway tissues is predominantly T cell derived. Immunocytochemical staining of bronchial biopsy sections has confirmed that IL-5 mRNA transcripts are translated into protein in asthmatic subjects. Furthermore, the number of activated CD 4 + T cells and IL-5 mRNA positive cells are increased in asthmatic airways following antigen challenge and studies that have examined IL-5 expression in asthmatic subjects before and after steroids have shown significantly decreased expression following oral corticosteroid treatment in steroid-sensitive asthma but not in steroid resistant and chronic severe steroid dependent asthma. The link between T cell derived IL-5 and eosinophil activation in asthmatic airways is further strengthened by the demonstration that there is an increased number of  $\alpha$ IL-5R mRNA positive cells in the bronchial biopsies of atopic and non-atopic asthmatic subjects and that the eosinophil is the predominant site of this increased  $\alpha$ IL-5R mRNA expression. We have also shown that the subset of activated eosinophils that expressed mRNA for membrane bound  $\alpha$  IL5r inversely correlated with FEV1, whereas the subset of activated eosinophils that expressed mRNA for soluble  $\alpha$ IL5r directly correlated with FEV1. Hence, not only does this data suggest that the presence of eosinophils expressing  $\alpha$ IL-5R mRNA contribute towards the pathogenesis of bronchial asthma, but also that the eosinophil phenotype with respect to  $\alpha$ IL-5R isoform expression is of central importance. Finally, there are several animal, and more recently in vitro lung explant, models of allergen induced eosinophilia, late airway responses (LARS), and bronchial hyperresponsiveness (BHR) - all of which support a link between IL-5 and airway eosinophils and bronchial hyperresponsiveness. The most direct demonstration of T cell involvement in LARS is the finding that these physiological responses can be transferred by CD4+ but not CD8+ T cells in rats. The importance of IL-5 in animal models of allergen induced bronchial hyperresponsiveness has been fur-*

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ther demonstrated by a number of studies which have indicated that IL-5 administration is able to induce late phase responses and BHR and that anti-IL-5 antibody can block allergen induced late phase responses and BHR.

In summary, activated T lymphocytes, IL5 production and eosinophil activation are particularly important in the asthmatic response. Human studies in asthma and studies in allergic animal models have clearly emphasised the unique role of IL-5 in linking T lymphocytes and adaptive immunity, the eosinophil effector cell, and the asthma phenotype. The central role of activated lymphocytes and eosinophils in asthma would argue for the likely therapeutic success of strategies to block T cell and eosinophil activation (eg steroids). Importantly, more targeted therapies may avoid the complications associated with steroids. Such therapies could target key T cell activation proteins and cytokines by various means including blocking antibodies (eg anti-CD4, anti-CD40, anti-IL-5 etc), antisense oligonucleotides to their specific mRNAs, and/or selective inhibition of the promoter sites for these genes. Another option would be to target key eosinophil activation mechanisms including the  $\alpha$ IL5r. As always, the risk to benefit ratio of such strategies await the results of well conducted clinical trials.

Key words: interleukin 5 - asthma - eosinophils

## ASTHMA AND ALLERGIC INFLAMMATION

Asthma is characterized by episodic airway obstruction, increased bronchial responsiveness to the inhalation of non-specific irritants and airway inflammation (McFadden & Gilbert 1992). The link between abnormal airway physiology and airway inflammation was initially suggested by the results of post-mortem studies of asthmatic lungs which documented the presence of an inflammatory infiltrate (Dunnill 1960). The use of fiberoptic bronchoscopy has allowed bronchial biopsies and lavage fluid to be examined in less severe asthmatics and in normal subjects. These studies have shown that eosinophils and T lymphocytes in particular are increased in number and activation status in asthmatic airways compared to controls (Jeffery et al. 1989, Azzawi et al. 1990, Bradley et al. 1991, Bentley et al. 1992, Laitinen et al. 1993). Furthermore, several studies have correlated the number of activated T cells and eosinophils with abnormalities in FEV1 and airway reactivity (Walker et al. 1991) and with clinical severity in asthma (Bousquet et al. 1990).

More recently, lung resection studies have also demonstrated an increase in the number of eosinophils and T lymphocytes in both the large and small airways of asthmatic subjects compared to non-asthmatic controls matched for age, sex, smoking history, lung function and airway size (Hamid et al. 1996). These results extend the findings from previous studies using endobronchial biopsies by showing that a similar but more severe inflammatory process is present in the peripheral airways and in the airway wall external to the smooth muscle layer-both of which are not routinely biopsied during fiberoptic bronchoscopy. This data is consistent with the evidence that suggests that the small airways are the major site of obstruction

in asthma (Macklem et al. 1970, Wiggs et al. 1992, Kuwano et al. 1993). In addition, the extensive presence of inflammatory cells throughout the airways makes it possible that these cells may be important modulators of the function of other cells present in airway tissues-including epithelial cells, fibroblasts and smooth muscle cells. There is therefore considerable interest and research into the mechanisms underlying the initiation and maintenance of the inflammatory response in asthma-which is likely to be a consequence of a complicated interaction between various cells and mediators.

## IL-5: AN IMPORTANT LINK BETWEEN T CELLS AND EOSINOPHILS

### IL-5 MOLECULAR BIOLOGY IL-5 GENE, MRNA AND PROTEIN

The human IL-5 gene is located on chromosome 5 in a gene cluster that contains the IL-4 family of cytokine genes (Boulay & Paul 1992). It contains 4 exons which encode a peptide of 124 amino acids (Azuma et al. 1986). IL-5 is a monomer which exists functionally as an antiparallel homodimeric glycoprotein linked by 2 disulfide bonds and has a tertiary crystalline structure consisting of 4 alpha helices (Milburn et al. 1993). The exon structure, primary secondary and tertiary protein sequences, cell membrane receptors and intracellular signal transduction pathways of IL-5 are similar with those from the IL-4 cytokine family, thereby suggesting that they are evolutionary related cytokines (Milburn et al. 1993, Kosugi et al. 1995). Therefore, it is not surprising that these cytokines also share cellular sources and functional activities and are all important in the co-ordinated immune defense against parasitic infection. However, IL-5 is unusual in that it is the most highly conserved member of this group. In addition, the IL-5 glycoprotein is highly homolo-

gous between mammalian species thereby suggesting that IL-5 function is of particular benefit to the host organism.

#### IL-5 RECEPTOR GENE, MRNA AND PROTEIN

The human IL-5 receptor complex is a heterodimer consisting of a unique  $\alpha$  subunit ( $\alpha$ IL-5R; MW 60Kd) and a  $\beta$  subunit (MW 130Kd) which is shared between the receptors for IL-3 and GM-CSF (Lopez et al. 1991, Murata et al. 1992, Miyajima et al. 1993). In vitro, the expression of human  $\alpha$ IL-5R has been described to be present on eosinophils and basophils whereas the  $\beta$  subunit is more widely expressed (Denburg et al. 1991, Migita et al. 1991, Miyajima et al. 1993). The  $\alpha$  subunit is required for ligand-specific binding whereas association with the  $\beta$  subunit results in increased binding affinity (Takagi et al. 1995). The receptors for IL-5, IL-3 and GM-CSF belong to the class I cytokine receptor family based on their structural motifs (Bazan 1990, Boulay & Paul, 1992b). The membrane proximal region of the extracellular domains of both the  $\alpha$  and  $\beta$  subunits of the IL-5R have common structural features which they share with the other members of the haematopoietin cytokine receptor family. This homologous region is characterized by a trp-ser-x-trp-ser motif and by several conserved short sequence elements - the integrity of which is required for the interaction with its ligand (Bazan 1990, Boulay & Paul 1992b).

The gene for human  $\alpha$ IL-5R is located on chromosome 3 (Tuypens et al. 1992) and the gene for human  $\beta$ IL-5R is on chromosome 22 (Miyajima et al. 1993, Takai et al. 1994). The alternative splicing of the  $\alpha$ IL-5R gene which contains 14 exons can yield several  $\alpha$ IL-5R isoforms including a membrane-anchored isoform ( $\alpha$ IL-5Rm) and a soluble isoform ( $\alpha$ IL-5Rs) (Tuypens et al. 1992, Tavernier et al. 1992). The membranous and soluble  $\alpha$ IL-5R isoform primarily differ in whether or not a transmembrane binding domain is present. Although,  $\alpha$ IL-5Rm and  $\alpha$ IL-5Rs isoform bind IL-5 with equally high affinity (Tavernier et al. 1992, Devos et al. 1993, Koike et al. 1994),  $\alpha$ IL-5Rm interacts with the  $\beta$  subunit thereby substantially increasing the affinity for IL-5 and allowing specific signal transduction pathways to be activated (Koike et al. 1994) whereas  $\alpha$ IL-5Rs competes for IL-5 ligand with  $\alpha$ IL-5Rm present on eosinophils and therefore has antagonistic properties that may have a regulatory role (Tavernier et al. 1991).

#### IL-5 RECEPTOR MEDIATED INTRACELLULAR SIGNAL TRANSDUCTION

Cytokines produce specific and non-specific cellular responses through receptor mediated acti-

vation of intracellular signal transduction pathways which, to a large part, regulate gene expression (Nicola et al. 1989, Miyajima et al. 1992). IL-5R mediated signalling requires the cytoplasmic domains of both subunits, is dependent on the proline rich areas proximal to the transmembrane domains and involves the process of dimerization of the  $\alpha$  and  $\beta$  subunits (Sakamaki et al. 1992, Miyajima et al. 1992, Takaki et al. 1993). The major intracellular signal transduction mechanism is activation of non-receptor associated tyrosine kinases including MAP kinases (Matsumoto et al. 1995, Pazdrak et al. 1995) and JAKS (Sakamaki et al. 1992, Corneils et al. 1995) which can then transduce signals via a novel family of transcriptional factors named signal transducers and activators of transcription (STATS) (Ihle et al. 1995a,b). STAT proteins exist in the cytoplasm as latent, transcriptionally inactive forms until in response to extracellular signals, they become phosphorylated on tyrosine residues, translocate to the nucleus, and bind to specific DNA elements. JAK2, STAT1 and STAT 5 appear to be particularly important in IL-5 mediated eosinophil responses (Mui et al. 1995, Van der Bruggen et al. 1995). Despite major advances in this area in recent years, the exact mechanisms by which IL-5 dependent, cell-type specific signals are generated are still to be elucidated.

#### IL-5, T CELLS AND EOSINOPHILS

Eosinophils, along with mast cells are the key cells involved in the innate immune response against parasitic infection. The adaptive immune response however is largely dependent on lymphocytes. CD4 +ve T lymphocytes, in particular, are crucial in antigen-driven inflammatory processes and are therefore likely to have an important role in orchestrating specific inflammatory responses. These cells are capable of recognizing foreign antigen that has been processed by antigen presenting cells and can produce pro-inflammatory cytokines in response to such activation which can dramatically amplify the inflammatory response.

One of the major links between T cells and eosinophils is IL-5. In chronic parasitic disease and in chronic allergic disease, IL-5 is predominantly a T cell derived cytokine whose major site of action is the eosinophil (Hamid et al. 1991, Mahanty et al. 1993, Ying et al. 1995). Although the development of tissue eosinophilia is T cell dependent, non T cell derived IL-5 may also play an important role as IL-5 mRNA can also be produced by mast cells and eosinophils (Plaut et al. 1989, Brodie et al. 1992). IL-5, IL-3, and GM-CSF are all capable of stimulating the development of eosinophils from human bone marrow. However, only IL-

5 was selective for the eosinophil lineage (Clutterbuck et al. 1989). Transgenic mice which constitutively express IL-5 have high level, life-long eosinophilia (Dent et al. 1990) and the administration of anti-IL-5 neutralising antibody in parasite infected mice totally blocks the production of eosinophilia (Coffman et al. 1989, Egan et al. 1995).

IL-5 is particularly important for the terminal differentiation of committed eosinophil precursors (Clutterbuck et al. 1989, Weller et al. 1992, Ogawa 1994). It activates mature eosinophils and prolongs their survival in culture (Yamaguchi et al. 1988)-possibly via its ability to delay apoptosis (Yamaguchi et al. 1991), as well as selectively enhancing eosinophil degranulation, antibody-dependent cytotoxicity and adhesion to vascular endothelium (Lopez et al. 1988, Fujisawa et al. 1990). IL-5 enhances the capacity of eosinophils to release LTC<sub>4</sub> (Weller et al. 1992) and also primes basophils, leading to increased histamine and LTC<sub>4</sub> generation (Bischoff et al. 1990, Laviollette et al. 1995) and increases synthesis of IgM, IgA, IgE by B cells costimulated with IL-4 (Pene et al. 1988, Purkerson & Isakson 1992). Although IL-5 on its own is minimally chemoattractant for eosinophils, its ability to significantly enhance the properties of stronger eosinophil chemoattractants such as Rantes and Eotaxin is probably more important (Sanderson, 1992, Sedgwick et al. 1995, Collins et al. 1995, Rothenberg et al. 1996).

#### IL-5, THE TH2 CYTOKINE PROFILE AND ALLERGIC INFLAMMATION

The production of IL-5 by T cells, like that of other TH2 cytokines, is independently regulated (Kelso, 1995, Naora et al. 1995, Sewell et al. 1996). Although individual T cells have the capacity to produce a wide range of cytokines, distinct T cell populations and cytokine profiles exist in chronic allergic inflammatory diseases (Miyajima et al. 1992, Van Straaten et al. 1994, Kay et al. 1995). There are a number of potential explanations for this phenomenon. Firstly, as has already been mentioned, the IL-4 family of cytokine genes is clustered on chromosome 5, have related evolutionary pathways and are therefore likely to be regulated by similar factors. Secondly, these factors are likely to co-exist in particular microenvironments - particularly when inflammation is driven by similar aetiological agents. And thirdly, these family of cytokines tend to upregulate themselves and downregulate opposing groups of cytokines in an attempt to generate a specific type of adaptive immune response (Modlin et al. 1993, Jung et al. 1995). Indeed, cross-regulation of T helper cell populations occurs and, in the extreme case, this

may lead to the development of relatively homogeneous Th1 and Th2 cell T cell population phenotypes (Kelso 1995).

Th2 cell populations tend to produce IL-4, 5, 13 and are associated with humoral immunity and allergy whereas TH1 cell populations tend to produce IFN- $\gamma$  and IL-2 and are associated with cell mediated immunity (Modlin et al. 1993). IL-4 and IL-13 are the cytokines that predominantly regulate B cell production of IgE and IgE activation of mast cells, both of which have an important role in the allergic immune response. The contribution of IL-4 and IL-5 to allergen induced eosinophil infiltration into the airway has been suggested by experiments showing inhibition of airway eosinophilia in mice with monoclonal antibodies directed against IL-4 and IL-5 (Moser et al. 1992, Kung et al. 1995). These cytokines may act as chemotactic factors for eosinophils, and also promote eosinophil-endothelial adhesion by inducing expression of VCAM-1 on endothelial cells. VCAM-1 in turn may bind to its receptor VLA-4 on the eosinophils leading to the migration of eosinophils to sites of airway inflammation (Elices et al. 1990). However, as has already been mentioned, one of the key roles of IL-5 however is to regulate eosinophil activation, differentiation and survival. Thus, although IL-5 also helps in the activation of B cells (Noelle et al. 1992), its major role is to recruit and activate eosinophils which act in concert with mast cells and IgE producing B cells in the immune response against parasites and in pathophysiology of allergic disease. The relative importance of these pathways is likely to vary according to the specific 'allergic' disease state. Nevertheless, the correlations between detectable levels of IL-5 mRNA in the tissues and IL-5 protein in the serum, eosinophilia development and disease pathology in a wide variety of allergic diseases are striking (including parasite infections, asthma, idiopathic eosinophilia, eosinophilic myalgia and Hodgkins lymphoma) (Sanderson 1992b). Hence, activated T cells can potentially initiate and propagate allergic inflammation in the airways and participate directly in the events responsible for asthma exacerbation by profoundly influencing both subsequent lymphocyte cell activation and the promotion of growth and differentiation of specific effector leucocytes such as eosinophils.

#### ASTHMA AND IL-5

##### HUMAN STUDIES: IL5 mRNA AND PROTEIN

*Atopic asthma* - It has now been well documented that IL-5 is highly expressed in the bronchial mucosa of atopic asthmatics and that the increased IL-5 mRNA present in airway tissues is predominantly T cell derived although fewer, but

detectable, numbers of tryptase+ mast cells and EG2+ eosinophils also expressed these transcripts (Hamid et al. 1991, Robinson et al. 1992, Kay et al. 1995, Ying et al. 1995). Immunocytochemical staining of bronchial biopsy sections has confirmed that IL-5 mRNA transcripts are translated into protein in asthmatic subjects (Fukuda et al. 1994).

Increased IL-5 mRNA expression has also been demonstrated in BAL T lymphocytes (Robinson et al. 1992) and in peripheral blood CD4 T cells (Corrigan et al. 1995). The reports of increased IL-5 protein levels in the BAL fluid, serum and peripheral blood T cell supernatants of asthmatics (Walker et al. 1992, 1991, Motojima et al. 1993), support the findings of increased numbers of IL-5 mRNA positive cells in these biological fluids. In addition, T cell lines that have been established from the BAL fluid and peripheral blood of atopic asthmatics secrete increased levels of IL-5 compared to atopic and non-atopic controls, thus providing further evidence that T cells in asthma have a propensity to make both IL-5 mRNA transcripts and IL-5 translated product which can then activate eosinophils (Endo et al. 1993, Okudaira et al. 1995, Till et al. 1995).

Numerous studies have shown that not only is there increased IL-5 mRNA and protein present in asthma, but also that the increased IL-5 is associated with increased eosinophil numbers and increased airways dysfunction (Hamid et al. 1991, Robinson et al. 1993, Sur et al. 1995). It has been demonstrated that the number of IL-5 mRNA-positive cells correlates with the number of eosinophils infiltrating the bronchial mucosa of asthmatic subjects and that IL-5 expression inversely correlates with pulmonary function (Hamid et al. 1991). In addition, the increased expression of IL-5 mRNA in BAL has been directly correlated to asthma symptom severity and inversely correlated to abnormal airway physiology (Robinson et al. 1993). Finally, in the studies where increased IL-5 protein levels were demonstrated in the BAL fluid, serum and peripheral blood T cell supernatants (Walker et al. 1992, 1991, Motojima et al. 1993) of asthmatic subjects, the IL-5 protein levels detected correlated with the numbers of eosinophils present in these fluids (Walker et al. 1992).

Activated eosinophils have the capacity to produce effector molecules that could participate in the pathogenesis of asthma. The demonstration of eosinophil major basic protein and eosinophil derived neurotoxin indicating degranulation at sites of injury are an important part of the evidence that eosinophils are producing tissue damage in the asthmatic lung (Sur et al. 1995). Hence, the current evidence suggests that the local production of IL-5 in asthmatic airways may play an important

role in the priming of eosinophils for subsequent activation, and in enhancing their survival at sites of allergic inflammation (Lopez et al. 1988, Yamaguchi et al. 1988), all of which is likely to be important in asthma.

*Intrinsic asthma* - Unlike extrinsic asthma, intrinsic asthma usually starts in adulthood, is perennial and is not atopy associated. Nevertheless, an analysis of the inflammatory cell populations present in both BAL fluid and bronchial biopsies from intrinsic asthmatics shows an increase in the number of activated T lymphocytes and eosinophils (Bentley et al. 1992). Indeed, there a large similarities in the inflammatory cells that are present in asthma of diverse aetiology-extrinsic, intrinsic, and occupational (Bentley et al. 1994). Moreover, several studies have now demonstrated increased levels of IL-5 mRNA and protein in the tissue and BAL fluid of intrinsic asthmatics, thereby supporting the role of this cytokine in both intrinsic as well as atopic asthma (Marini et al. 1992, Bentley et al. 1993, Walker et al. 1994, Humbert et al. 1996). These findings support a common T cell mediated basis for airway inflammation in both forms of asthma.

The difference between intrinsic and atopic asthma is the lack of demonstrable specific IgE to an antigen in individuals with intrinsic disease. Although total serum IgE levels have been noted to be increased in the serum of patients with intrinsic asthma (Burrows et al. 1989) this is not a uniform finding with high positive predictive value (Butcher et al. 1980, Klink et al. 1990). There is evidence that IL-5 but not IL-4 is increased in the BAL fluid from intrinsic asthmatics (Walker et al. 1994), thus supporting the hypothesis that IL-5 and eosinophilia are key features in both forms of asthma and that differences in the type of cytokine synthesis may undermine the differences in immune pathology that exist between intrinsic and atopic asthma. However, more recent studies examining the expression of high-affinity IgE receptor (Humbert et al. 1996a), and IL-5 and IL-4 mRNA and protein expression in bronchial biopsies from patients with atopic and non-atopic asthma found no difference between atopic and intrinsic asthmatics (Humbert et al. 1996b). Thus, any differences in immune pathology that may exist between intrinsic and atopic asthma may be more subtle than initially expected.

*Other allergic pulmonary and non-pulmonary allergic diseases* - There is a strong association between IL-5 and eosinophilia in a number of allergic human diseases, including parasitic infections (Coffman et al. 1989, Limaye et al. 1993, Hagan et al. 1996), atopic dermatitis (Frew & Kay 1988,

Hamid et al. 1994), eosinophilic myocarditis (Desreumaux et al. 1993), hypereosinophilic syndrome (Schrezenmeier et al. 1993, Satoh et al. 1994) eosinophilic gastroenteritis (Quan et al. 1993, Dubucquoi et al. 1995), allergic rhinitis (Durham et al. 1992), chronic eosinophilic pneumonia (Kita et al. 1996) and other eosinophilic lung diseases (Walker et al. 1994) (Fig. 1).

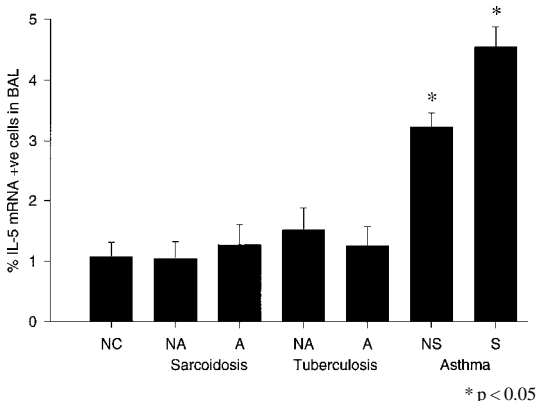


Fig. 1: IL-5 in asthma and other pulmonary diseases. The percentage of IL-5 mRNA +ve cells in the BAL fluid of patients with asthma, sarcoidosis, and tuberculosis. NC: normal controls; NA: non-active disease; A: active disease; NS: non-symptomatic disease; S: symptomatic disease.

Although there is a strong link between IL-5 and eosinophilia in general, the link between pulmonary eosinophilia and clinical asthma is less direct. Non-eosinophilic inflammatory lung conditions such as tuberculosis and sarcoidosis are not associated with increased IL-5 and eosinophils (Taha et al. 1996, Minshall et al. 1996). However, although eosinophilia is a common feature of asthma many eosinophilic lung diseases are not associated with clinical asthma. This argues that factors other than the presence of eosinophils are also important in the development of clinical asthma. These factors may relate to the level of eosinophil activation, to non-eosinophil dependent parameters that are nevertheless associated with allergic inflammation, or to baseline levels of bronchial hyperresponsiveness. It is therefore likely that the asthma phenotype is most likely to occur when all the relevant factors- including IL5 and eosinophilia, occur together in an individual predisposed to bronchial hyperresponsiveness.

**Antigen challenge** - The number of activated CD 4 + T cells and IL-5 mRNA positive cells are increased in asthmatic airways following antigen challenge (Robinson et al. 1993, Bentley et al. 1993) (Fig. 2). Furthermore, CD 4 + ve T cells have been generally implicated as the major IL-5 mRNA positive cell present following antigen chal-

lenge in atopic asthmatics (Bentley et al. 1993, Robinson et al. 1993), although some investigators have reported that the eosinophil is also a source of IL-5 in this setting (Broide et al. 1992). Studies examining BAL samples 18-48 hr after allergen challenge have also shown increased expression of IL-5 (Krishnaswamy et al. 1993, Ohnishi et al. 1993). In addition, IL-5 was a major cytokine product of T cells from patients with mite associated bronchial asthma when they were stimulated with *Dermatophagoides farinae* (Kamei et al. 1993).

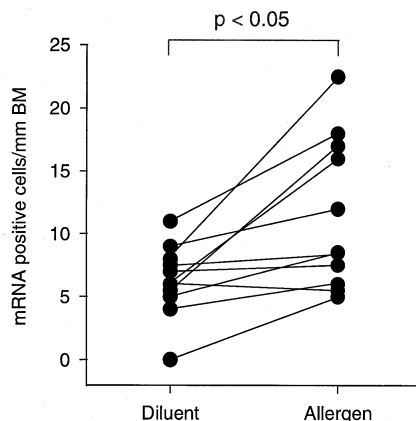


Fig. 2: IL-5 mRNA following allergen challenge humans. The number of IL-5 mRNA +ve cells/mm basement membrane in atopic asthmatic subjects following specific allergen challenge.

The increased IL-5 expression that follows allergen challenge has been demonstrated to inversely correlate with pulmonary function (Bentley et al. 1993) and this adds to the evidence that IL-5 expression and eosinophilia are relevantly increased following exposure to antigen in sensitised individuals. Indeed, in the study by Ohnishi et al. (1993) a segmental antigen lung challenge model was used to show that IL-5 was the most important constituent increasing eosinophil survival and that IL-5 correlated with eosinophil recruitment, degranulation and lung injury following inhalation of antigen. These results are in agreement with several other studies that have indicated that increases in the levels of eosinophils and their cationic proteins in the BAL fluid following allergen challenge correlates with the magnitude of the late phase response (Pradalier 1993).

Furthermore, it is well recognized that there is an association between allergic rhinitis and allergic asthma, and hence studies using models of allergen-induced allergic rhinitis are therefore relevant to allergic asthma. The results obtained from such models by and large support the above

findings. For instance, it has been shown that T cells are the principal source of IL-5 transcripts in the nasal mucosa following allergen induce late-phase nasal responses (Ying et al.1993). Similar findings have also been reported in models of allergen induced cutaneous late phase reactions (Kay et al. 1991).

**Steroid treatment** - The use of anti-inflammatory corticosteroids are the cornerstone of current asthma therapy. They have been shown to be extremely effective clinically. Studies that have examined IL-5 expression in BAL and peripheral blood of asthmatic subjects before and after steroids have shown that the number of IL-5 mRNA positive cells is significantly decreased following oral corticosteroid treatment in steroid-sensitive asthma (Robinson et al. 1993, Corrigan et al. 1995). In contrast, steroid resistant asthma and chronic severe steroid dependent asthma are associated with persistently elevated IL-5 mRNA levels (Leung et al. 1995) and serum IL-5 levels (Alexander et al. 1994), respectively . The decreases in the expression of IL-5 that followed corticosteroid therapy have been associated with decreased eosinophil numbers - especially in the peripheral blood (Corrigan et al. 1995), but increased numbers of IFN- $\gamma$  positive cells in the bronchial mucosa and BAL fluid of asthmatic subjects (Robinson et al. 1993, Leung et al. 1995, Bentley et al. 1996). These findings support the direct link between IL-5 and eosinophils and the inverse relationship between Th1 and Th2 type T cells in asthma (Fig. 3). Hence, corticosteroid treatment in asthma may act by modulation of cytokine expression with consequent inhibition of the local bronchial inflammatory infiltrate and tissue eosinophilia.

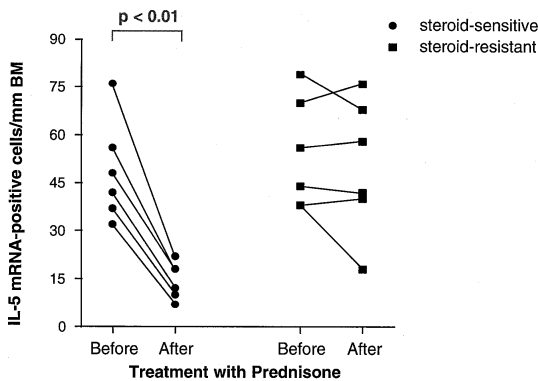


Fig. 3 : prednisone therapy and IL-5 mRNA expression in steroid-sensitive and steroid-resistant asthmatics. The number of IL-5 mRNA +ve cells/mm basement membrane in the bronchial mucosa before and after prednisolone therapy in steroid-sensitive and steroid-resistant asthmatics.

**IL5 RECEPTOR (Membrane bound and soluble isoforms)**

The link between T cell derived IL-5 and eosinophil activation in asthmatic airways has now been supported by the demonstration that there is an increased number of  $\alpha$ IL-5R mRNA positive cells in the bronchial biopsies of atopic and non-atopic asthmatic subjects compared with atopic and non-atopic controls (Fig. 4a , b) and that the eosinophil is the predominant site of this increased  $\alpha$ IL-5R mRNA expression (Yasruel et al. 1997). We have shown that 93% of the  $\alpha$ IL-5R mRNA positive cells within the bronchial mucosa of asthmatics were also EG2 positive thereby suggesting that IL-5 may play an important local role in stimulating eosinophils via the specific  $\alpha$ -subunit of its receptor. These results support previous work that has suggested that the lineage specificity of IL-5 is mainly due to the restricted expression of the  $\alpha$  subunit of IL5R (Takagi et al. 1995) and demonstrates that the expression of  $\alpha$ IL-5R in vivo can be much more cell-restricted than that seen in vitro (Lopez et al. 1991).

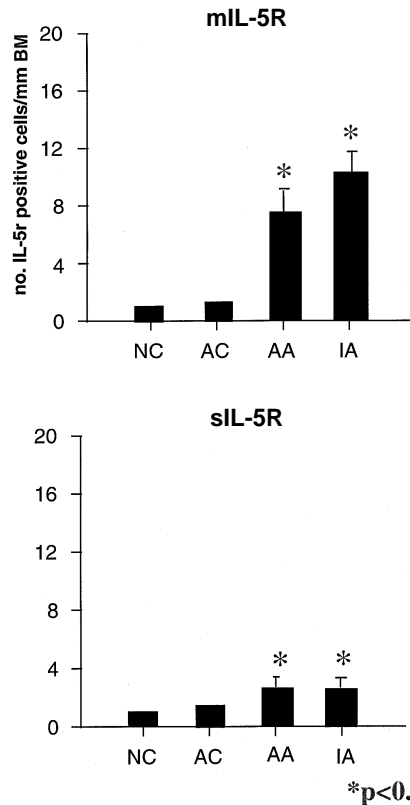


Fig 4 a/b: membrane and soluble IL-5R mRNA expression in asthma. The number of membrane and soluble IL-5 receptor mRNA +ve cells/mm basement membrane in the bronchial mucosa in asthma. mIL-5r: membrane-bound IL-5r isoform; sIL-5r: soluble IL-5r isoform; NC: normal controls; AC: atopic controls; AA: atopic asthmatics; IA: intrinsic asthmatics.

We have also shown that the subset of activated eosinophils that expressed mRNA for membrane bound IL5r inversely correlated with FEV<sub>1</sub>, whereas the subset of activated eosinophils that expressed mRNA for soluble IL5r directly correlated with FEV<sub>1</sub>. Hence, not only does this data suggest that the presence of eosinophils expressing IL-5R mRNA contribute towards the pathogenesis of bronchial asthma, but also that the eosinophil phenotype with respect to  $\alpha$ IL-5R isoform expression is of central importance. Factors that may modulate the activation phenotype of the airway eosinophils in asthma remain to be clearly determined. The apparent contradiction between an EG2+ eosinophil (EG2 being a marker of activated eosinophils) also expressing  $\alpha$ IL-5Rs mRNA and hence representing a downregulated cell can be explained by assuming that EG2 and the  $\alpha$ IL5R are associated with different levels of eosinophil activation. Indeed, there is considerable controversy as to the validity of EG2 as a marker of eosinophil activation (Moqbel et al. 1992). Moreover, there is no direct way with which to grade the various potential activation of eosinophils. If EG2 represented a relatively low grade eosinophil activation marker then it is possible that when eosinophils cross the endothelial barrier they all become EG2 + as a result of influences from the local tissue environment. On the other hand,  $\alpha$ IL-5R activation status might represent a higher level of cell activation such that  $\alpha$ IL-5Rm positive cells represented a highly activated subset of EG2+ eosinophils and  $\alpha$ IL-5Rs positive cells a subset of EG2+ eosinophils that are minimally activated. Hence, although previously published correlations between EG2+ eosinophils and FEV<sub>1</sub> were only modest (Hamid et al. 1991), such considerations may explain the strong inverse correlation between  $\alpha$ IL-5Rm mRNA positive cells and FEV<sub>1</sub> as well as the strong direct correlation between  $\alpha$ IL-5Rs mRNA positive cells and FEV<sub>1</sub> that was reported.

The central question as to what controls the transcriptional regulation of  $\alpha$ IL-5R also remains to be determined. Transforming growth factor B1 has already been shown to downregulate  $\alpha$ IL-5R mRNA expression (Zanders 1994), however the effect of single cytokines or combinations of cytokines on the production of different mRNA splice variants of  $\alpha$ IL-5R is yet to be elucidated. Nevertheless, the increased number of  $\alpha$ IL-5R mRNA positive eosinophils in the bronchial tissue of asthmatic patients and the differential expression of  $\alpha$ IL-5R mRNA isoforms in atopic and non-atopic asthma support the central roles of IL-5 and eosinophils in the pathobiology of asthma.

#### IL5 ASSOCIATED SIGNAL TX AND GENE ACTIVATION

It has been shown that cloned human naive CD4 T cells develop into IL-4 and IL-5 producing effector cells as a default pathway (Yang et al. 1995). It could therefore be hypothesised that inert antigenic stimulation of the immune system without concomitant stimulation of cell mediated immune pathways would favour the development of allergic responses. However, how exactly allergen induced activation of TCR and co-stimulatory molecules translates to IL-4 and IL-5 gene activation is not clear. It could also be hypothesised that it would be in the interests of a well coordinated amplification cascade of inflammation to link IL-4 and IL-5 gene activation in cytokine producing cells with IL-4 and IL-5 receptor gene activation in target cells. Although we have already quoted some evidence that suggests that this occurs, the exact mechanisms are unclear.

The functions of the alpha subunits of IL-5R and IL-4R have been examined by co-transfecting human cDNAs for these subunits into human cell lines, and it is clear that intracellular signalling is very different in both cases (Chen et al. 1994). How IL-4 mediated intracellular signals interact with the IL-5 gene promoter and whether IL-4 and IL-5 mediated signal transduction can also increase the expression of IL-4 and IL-5 cytokines from source cells and IL-4 and IL-5 receptors in target cells are important issues still to be elucidated. Furthermore, how IL-5 mediated signals translates to an activated eosinophil phenotype is also unclear at present, although recent work has implicated specific GATA binding proteins (Zon et al. 1993). Finally, the response of these cytokine and cytokine receptor genes to therapeutic agents is another important area requiring further study.

#### ANIMAL STUDIES

##### IL-5 EXPRESSION: SENSITISED AND ANTIGEN CHALLENGED ANIMALS

There are several animal models of allergen induced eosinophilia, late airway responses, and bronchial hyperresponsiveness. These include guinea pigs (Corry et al. 1996), Brown Norway (BN) rats (Renzi et al. 1991a,b, 1993, Olivenstein et al. 1993) and mice (Nakajima et al. 1992, Iwamoto et al. 1992). In all these models there is evidence to support a link between IL-5 and airway eosinophilia and bronchial hyperresponsiveness. In the BN rat we have also shown that CD4+ve T cells and Th-2 cytokines, IL-5 in particular, are involved in allergen induced late airway responses (LAR) (Fig. 5) (Al Assad et al. 1995, Renzi et al. 1996).



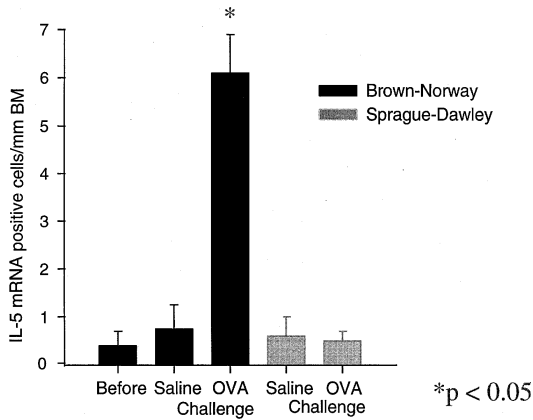


Fig 5: IL-5 mRNA expression following allergen challenge in BN rats. The number of IL-5 mRNA +ve cells/mm basement membrane in the bronchial mucosa of Brown Norway rats and Sprague-Dawley rats following ovalbumin allergen challenge.

We have shown that the airways of OVA sensitized BN rats are infiltrated predominantly by IL-5 and IL-4 mRNA +ve cells after antigen challenge (Renzi et al. 1996). Eum et al. have demonstrated that eosinophil recruitment into the respiratory epithelium following antigenic challenge is associated with IL-5-dependent bronchial hyperresponsiveness (Eum et al. 1995). Recent work has shown that IL-5 deficiency abolishes eosinophilia, airways hyperreactivity and lung damage in a mouse asthma model and that reconstitution of IL-5 production using recombinant vaccinia virus that expressed IL-5 restored aeroallergen induced eosinophilia and airways dysfunction (Foster et al. 1996). IL-5 transgenic mice show marked eosinophilia and increased reactivity to acetylcholine only after antigen challenge. This suggests that eosinophil activation rather than just large numbers of eosinophils is crucial to the development of BHR (Iwamoto et al. 1995). Interestingly, genetic linkage analysis has linked bronchial hyperresponsiveness in the mouse to murine chromosome 6 - the chromosomal region containing the gene for IL-5 (Ewart et al. 1996).

Using an in vitro lung explant model it has also been shown that airways of OVA sensitized BN rats are infiltrated predominantly by MBP + ve, IL-5 and IL-4 mRNA +ve cells after ex-vivo antigen challenge (Fig. 6) (Minshall et al. 1996). The demonstration of increased MBP and IL-5 mRNA expression in sensitized lung explants after allergen challenge, suggests that local factors are likely to be very important in the initiation and development of airway eosinophil infiltration.

The most direct demonstration of T cell involvement in LARs is the finding that these physi-

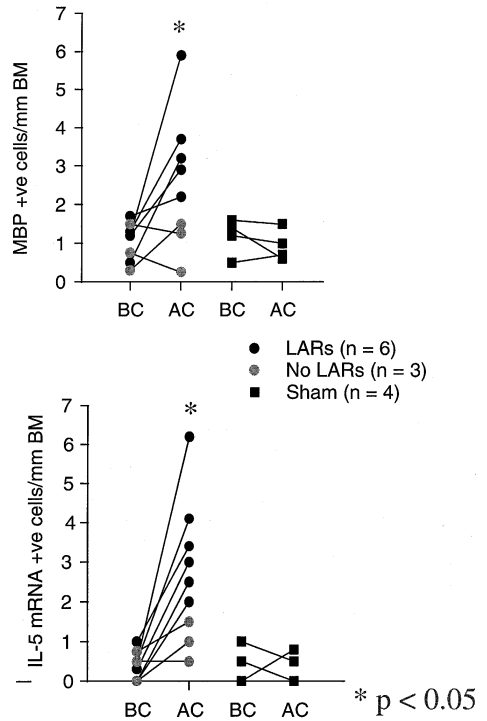


Fig 6: MBP and IL-5 mRNA expression in sensitized BN rat lung explants. The number of MBP and IL-5 mRNA +ve cells/mm basement membrane in sensitized Brown Norway rat lung explant tissue before and after ovalbumin allergen challenge. LAR: late phase airway response; BC: before challenge; AC: after challenge.

ological responses can be transferred by CD4+ but not CD8+ T cells in rats (Watanabe et al. 1995a,b). To investigate the role of T cell cytokines in these responses the expression of mRNA for Th2 (IL-4 and IL-5) and Th1 (IL-2 and INF- $\gamma$ ) type cytokines in BN rats that were administered aerosolized OVA challenge following the adoptive transfer of either antigen-primed W3/25(CD4)+ or OX8(CD8)+ T cells was examined (Fig. 7) (Watanabe et al. 1996). Our results showed that recipients of OVA-primed CD4+ T cells had an increase in the fraction of BAL cells expressing mRNA for IL-4 and IL-5 compared to BSA-primed CD4+ or OVA-primed CD8+ cells. Recipients of CD8+ T cells had an increase in INF- $\gamma$  mRNA expression after OVA challenge compared to recipients of BSA primed CD8+ or OVA primed CD4+ T cells. Hence, T cell dependent allergen induced late responses are associated with the expression of mRNA for IL-4 and IL-5, indicating Th2 cell activation. Furthermore, the increased expression of INF- $\gamma$  in allergen challenge recipients of antigen-primed CD8+ T cells suggests

that CD8<sup>+</sup> T cells may be important in modulating allergic responses, thus supporting the conclusions from previous work in this area (Al Assad et al. 1995).

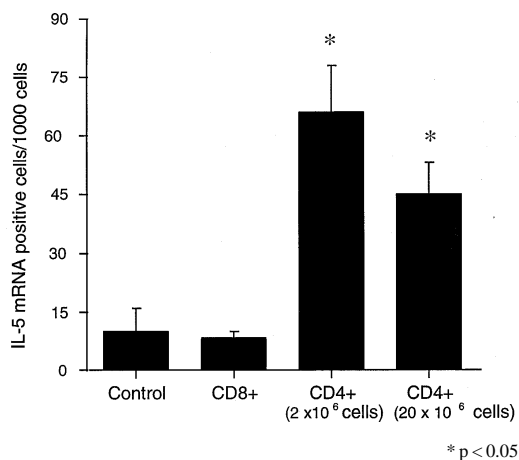


Fig 7: IL-5 mRNA-positive cells in BAL following adoptive transfer of T cells. The number of IL-5 mRNA +ve cells/1000 cells in the BAL fluid of OVA-challenged BN rats that were recipients of OVA-primed T cells (CD4<sup>+</sup> and CD8<sup>+</sup> groups).

#### IL-5 AND ANTI-IL-5 ADMINISTRATION

The importance of IL-5 in animal models of allergen induced bronchial hyperresponsiveness has been further demonstrated by a number of studies which have indicated that IL-5 administration is able to induce late phase responses and BHR and that anti-IL-5 antibody can block allergen induced late phase responses and BHR. IL-5 administration has been shown to increase mucosal exudation, enhance eosinophil recruitment into the lungs and to increase airways responsiveness in models of allergen induced BHR in the guinea pig (Gulbenkian et al. 1992, van Oosterhout et al. 1993a), in mice (van Oosterhout et al. 1993b), and in the Brown Norway rat (Renzi et al. 1996). Moreover, anti-IL-5 administration inhibits eosinophil recruitment and airways hyperresponsiveness in guinea pig models of allergic pulmonary inflammation and allergen induced BHR (Gulbenkian et al. 1992, van Oosterhout et al. 1993a, Das et al. 1995). Similar findings have also been demonstrated in the mouse (Nagai et al. 1993, Kung et al. 1995) and in the monkey (Mauser et al. 1995).

#### IL-5 SIGNAL TRANSDUCTION MODULATION

Many animal studies have now demonstrated that eosinophilia is a uniquely specific phenomenon regulated by IL-5 which of course suggest that IL-5 gene expression is under specific control. This control has been investigated by exam-

ining the roles of various transcription factors in animal T cell lines (Lee et al. 1994, Karlen et al. 1996). These studies have implicated AP-1, NF-AT like factors and GATA binding proteins although it is still not clear how all these molecules are related in controlling IL-5 promoter activity (Lee et al. 1994, Yamagata et al. 1995, Karlen et al. 1996).

#### THERAPEUTIC IMPLICATIONS

Asthma is a complex disorder involving a specific inflammatory response in the airways that is largely co-ordinated by activated T cells and involves various other inflammatory effector cells especially eosinophils but also B cells and mast cells, as well as functional and structural changes in the resident airway tissue cells. The central role of activated lymphocytes and eosinophils in asthma would argue for the likely therapeutic success of strategies to block T cell and eosinophil activation. It is likely that at least some of the success of steroids in suppressing asthmatic inflammation is due to their ability to suppress T lymphocyte and eosinophil activation. However, steroids have a wide range of effects on many other inflammatory and non-inflammatory cells. Although this may be the basis of the therapeutic usefulness of steroids, it is also the basis of the significant side effects associated with their long term use. Importantly, more targeted therapies may avoid the complications associated with steroids. Such therapies could target key T cell activation proteins and cytokines by various means including blocking antibodies (eg anti-CD4, anti-CD40, anti-IL-5 etc), antisense oligonucleotides to their specific mRNAs, and/or selective inhibition of the promoter sites for these genes. Another option would be to target key eosinophil activation mechanisms including the  $\alpha$ IL5r. As always, the risk to benefit ratio of such strategies await the results of well conducted clinical trials.

The groundwork for such strategies is currently being laid. Anti-IL5 antibodies have been shown to inhibit pulmonary eosinophilia, tissue damage and hyperactivity in allergic animal models (Egan et al. 1995, Hagan et al. 1996). It has also been demonstrated that FK506 could suppress IL-5 production and gene expression in a dose dependent manner (Okudaira et al. 1995). In addition, the data of Yasrael et al. (1996) linking soluble  $\alpha$ IL-5r expression with improved FEV<sub>1</sub> levels and studies examining the therapeutic potential of soluble  $\alpha$ IL-5r suggest that they may offer particular promise (Devos et al. 1995). Indeed, the possibility of the antagonistic properties of the  $\alpha$ IL-5Rs being used as a therapeutic option in eosinophil mediated disease states such as asthma and allergic rhinitis is

already being investigated (Zanders 1994, Devos et al. 1995), although the response of  $\alpha$ IL-5R expression to antigen challenge and steroid treatment are still important areas of further study. Interestingly, the potential biological modulatory role of  $\alpha$ IL-5Rs also has important implications for the development of IL-5R antagonists as these antagonists may not differentiate between binding to and inhibiting  $\alpha$ IL-5Rm, and binding to and inhibiting  $\alpha$ IL-5Rs (Devos et al. 1994, 1995). Furthermore, in a murine model of allergic responses, it has been shown that soluble  $\alpha$ IL-5r suppressed antigen induced BAL eosinophilia with little effect on airway hyperreactivity reminding us again of the complexities involved in all these responses (Yamaguchi et al. 1994).

## CONCLUSIONS

In summary, activated T lymphocytes, the production of IL-5 and eosinophil activation are particularly important in the asthmatic response. Human studies in asthma and studies in allergic animal models have clearly emphasised the unique role of IL-5 in linking adaptive immunity and T lymphocytes with the eosinophil effector cell. However, how this link between IL-5 producing T cells and IL-5 target eosinophils is initiated, propagated and attenuated is still an area that requires further research. In addition, what the exact activation characteristics of the T lymphocytes in asthma are (V $\beta$  restriction of their TCR in response to specific antigens) and what the relative effects of T cells and Th2 cytokines are on all effector inflammatory cells and on structural cells (epithelium, fibroblasts and smooth muscle cells) of asthmatic airways are also important issues that need to be resolved. It is hoped that answers to these questions in the near future will provide us with an increased understanding of asthma pathogenesis, and ultimately lead to novel, highly targeted and effective therapeutic strategies for asthma management.

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