Expression of Membrane-anchored and Soluble Isoforms of Interleukin-5 Receptor α mRNA in Bronchial Asthma

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ABSTRACT

Expression of Membrane-anchored and Soluble isoforms of Interleukin-5 Receptor α mRNA in Bronchial Asthma

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Interleukin-5 (IL-5) and eosinophils are closely associated in the pathogenesis of asthma. IL-5 promotes eosinophil growth, activation and survival in vitro. The α subunit of the IL-5 receptor (αIL-5R) is specific for IL-5 binding. Alternate processing of the αIL-5R gene transcript can yield either membrane bound or soluble isoforms (αIL-5Rm and αIL-5Rs). This thesis examines the differential expression of αIL-5Rm & αIL-5Rs, the phenotype of the cells expressing the αIL-5R receptor mRNA and correlates the expression of specific αIL-5R isoforms with the clinical features of asthma, characterised by airway obstruction (FEV_1) and airway hyperresponsiveness (methacholine PC_{20}). A significant increase in the number of cells/mm basement membrane expressing αIL-5Rm and αIL-5Rs mRNA was observed in asthmatics compared to non-asthmatic controls. There was no significant difference in the expression of αIL-5Rm and αIL-5Rs between atopic and non-atopic asthmatics. Ninety-three percent of the αIL-5R mRNA positive cells from asthmatics were activated eosinophils. The expression of αIL-5Rm mRNA was inversely correlated with the FEV_1, whereas the expression of αIL-5Rs mRNA was directly correlated with the FEV_1. There were no significant correlations between αIL-5R isoforms and PC_{20}. There was a significant inverse correlation between αIL-5Rm and αIL-5Rs mRNA. These findings support the role of IL-5 in regulating the eosinophil function associated with asthma. Furthermore, the enhanced

eosinophil expression of αIL-5R mRNA and the differential expression of αL-5R mRNA products may affect the inflammatory response in asthma and thereby increase airway obstruction

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ABBREVIATIONS

AA	atopic asthmatics
AHR	airway hyperresponsiveness
ANC	atopic normal control
APAAP	alkaline phosphatase anti-alkaline phosphatase
APC	antigen-presenting cells
ATP	adenosine-5'-triphosphate
BAL	broncoalveolar lavage
CD	cluster of differentiation
cDNA	complementary deoxyribonucleic acid
cRNA	complementary ribonucleic acid
CTP	cytidine-5'-triphosphate
DEPC	diethylpyrocarbonate
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
DTT	dithiothreitol
EAR	early asthmatic response
ECP	eosinophil cationic protein
EDN	eosinophil derived neurotoxin
EDTA	ethylenediaminetetraacetic acid
EPO	eosinophil peroxidase

FcR	receptors for the Fc portion of immunoglobulin
FEV_1	forced expiratory volume in 1 second
GM-CSF	granulocyte macrophage-colony stimulating factor
GTP	guanosine-5'-triphosphate
JAK2	janus kinase 2
IA	intrinsic asthmatics
ICC	Immunocytochemistry
IFN-γ	interferon-gamma
Ig	Immunoglobulin
IL	interleukin
αIL-5	interleukin-5 receptor alpha subunit
αIL-5Rm	interleukin-5 receptor alpha subunit membrane anchored form
αIL-5Rs	Interleukin-5 Receptor alpha subunit soluble form
ISH	*in situ* hybridisation
LAR	late asthmatic response
LT	leukotriene
mAb	monoclonal antibody
MAP	microtubule associated protein
MBP	major basic protein
MCP	monocyte chemoattractant protein
MHC	major histocompatibility complex
mRNA	messenger ribonucleic acid

NANC	nonatopic normal control
PAF	platelet activating factor
PBS	phosphate buffered saline
PC	provocative concentration
PGD	prostaglandin D
RANTES	regulated and normal T-lymphocyte expressed and secreted
PLL	poly-L-lysine
RNA	ribonucleic acid
RNase	ribonuclease
RT-PCR	reverse transcription polymerase chain reaction
SDS	sodium dodecyl sulphate
STAT	signal transducers and activators of transcription
SSC	saline sodium citrate
TBS	tris buffered saline
TGF	transforming growth factor
Th	T-helper cells
TNF	tumour necrosis factor
UTP	uridine-5'-triphosphate
VCAM-1	vascular endothelial adhesion molecule-1
VLA-4	very late antigen-4

DECLARATIONS

1) Bronchial biopsies from 19 asthmatics (atopics=9; intrinsics=10) and non-asthmatics (atopics=10; non-atopics=10) were obtained by Dr. M. Humbert using fiberoptic bronchoscopy from the Allergy Clinic at Royal Brompton National Heart and Lung Hospital, London, England. These biopsies were transferred in dry ice to Meakins-Christie Laboratories, McGill University, Montreal, Canada for processing for this thesis project.

2) The following cDNA fragments αIL5R were previously characterised and kindly provided by Dr. J. Tavernier, Roche Research, Brussels, Belgium to be used for this thesis work.

   a) 1074 bp non-specific cDNA recognising both soluble and membrane-anchored αIL5R was provided in pCDM8 at *Xba* I site.

   b) 84 bp cDNA for soluble specific αIL5R was provided in pCDM8 vector at *BstX* I site.

   c) 92 bp cDNA for membrane-anchored specific αIL5R was provided in pCDM8 vector at *BstX* I site.

1.0 INTRODUCTION

1.1 Asthma - definition

Asthma is defined as a disease of increased non-specific airway responsiveness with variable airway obstruction and airway inflammation (American Thoracic Society, 1987). This disease afflicts approximately 6% of the adult population. The clinical symptoms of asthma include airflow limitation, shortness of breath, and wheezing (McFadden and Gilbert, 1992). Airway obstruction is reversed either spontaneously or as a result of therapy although in some cases, the occlusion fails to reverse.

Histopathology of airways from patients who have died from asthmatic attacks depict detachment of epithelium, thickening of the basement membrane, contraction of airway smooth muscle, mucus hypersecretion and cellular infiltration, particularly of eosinophils and lymphocytes (Dunnill et al., 1969; Cutz et al., 1978). With the availability of fiberoptic bronchoscopy to obtain lavage and tissue samples from the airways, even from mildly affected patients, the mucosa has been confirmed to be abnormal *in situ*. Eosinophil infiltration of the lamina propria, thickening of the basement membrane and epithelial disruption have all been observed (Beasley et al., 1989). Airway remodelling, chronic inflammation, and enhanced airway constriction are thus considered hallmarks of asthma.

Recent attention has focused on the interaction between activated T cells and eosinophils because activated lymphocytes and eosinophils identified in bronchial tissue sections from mild asthmatics were shown to correlate with the severity of the disease (Azzawi

et al., 1990). Thus, chronic, persistent airway inflammation is now considered to be a significant feature of the pathophysiology of asthma.

1.2 Aetiology

Asthma was classically described as a disease mediated by Immunoglobulin E (IgE) antibody attached to mast cells in the airway mucosa of atopic patients (Ishizaka & Ishizaka, 1971). Atopy, or allergy, is characterised by the enhanced sensitivity to normal exposure of non-specific substances, or allergens, resulting in increased synthesis of IgE (Coca and Cooke, 1923).

Initial exposure to allergen initiates the sensitisation process of atopy. The allergen is degraded by macrophages and other antigen-presenting cells. Degraded fragments are presented to T lymphocytes which in turn help B lymphocytes to produce allergen-specific IgE antibodies. These antibodies attach to high affinity receptors (FcεRI) on tissue mast cells, causing degranulation and the release of histamine and other chemical mediators (Kinet, 1989). Once the IgE antibodies have been synthesised, their memory persists such that subsequent exposure to allergen causes immediate activation of mast cells.

Bronchial asthma which is mediated by sensitisation to inhaled allergens is described as being extrinsic. Extrinsic asthmatics respond to allergen inhalation with airway narrowing within fifteen minutes after exposure, followed by spontaneous resolution in one to two hours. This is described as the early asthmatic response (EAR) and is mediated by mast cell activation. Some patients experience a subsequent episode of airway narrowing within four to eight

hours after the initial allergen exposure that may last 12 to 24 hours. This is called the late asthmatic response (LAR) (O'Byrne et al., 1987) which is associated with a complex sequence of events which are characteristic of chronic asthma: increased mucus secretion, development of airway inflammation and particularly recruitment of eosinophils into the airways, and airway hyperresponsiveness (Walker et al., 1991a, 1992). Extrinsic asthma generally starts in childhood and associated with CD4+ T lymphocyte activation in blood and bronchoalveolar lavage (BAL) fluid (Robinson et al., 1992: Walker et al., 1992).

Contrary to extrinsic asthmatics, intrinsic asthmatics are non-atopic and are provoked by nonantigenic stimuli (Table 1). Intrinsic asthma is not well understood but usually starts during adulthood and is marked by the activation of both CD4+ and CD8+ T-lymphocytes in blood and BAL fluid (Walker et al., 1992)

Increases in the activated T lymphocytes in the peripheral blood, bronchial biopsies and BAL fluid have been demonstrated in both extrinsic and intrinsic asthma and this increase in activated T lymphocytes correlates closely with increased levels of eosinophils and disease severity (Walker et al., 1991a, 1991b, 1992; Bentley et al., 1992). However, they have distinct cytokine profiles (Fig. 1). Extrinsic asthmatics have enhanced levels of IL-4 and IL-5 immunoreactivity in BAL fluid while intrinsic asthmatics have detectable quantities of IL-5, IL-2, interferon-γ (IFN-γ) immunoreactivity but not IL-4 (Walker et al., 1992). The same study showed that eosinophil numbers in the BAL are also increased in both extrinsic and intrinsic asthma. Therefore in both extrinsic and intrinsic asthma, there are significant increases in the

level of IL-5 and eosinophil count, suggesting that IL-5 may be a specific cytokine involved in the eosinophil infiltration within the airways of these two disorders.

The clinical symptoms of and therapeutical approaches to extrinsic and intrinsic asthma are similar and are therefore generally described simply as asthma.

Table 1: Clinical Features of Extrinsic and Intrinsic asthma

(Adopted from Walker, 1993)

	Extrinsic asthma	**Intrinsic asthma**
Age of onset	Childhood	Adult
Gender	Males>Females	Females>Males
Symptomology	Seasonal Allergen induced	Perennial Unknown
Allergies	Multiple	None
Skin Prick test	Positive	Negative
Serum IgE	Elevated	Normal
Increased T cell type	CD4+	CD4+ and CD8+
Cytokines profile in BAL	IL-5 and IL-4	IL-5, IL-2 and IFN-γ
Eosinophil number in BAL	High	High

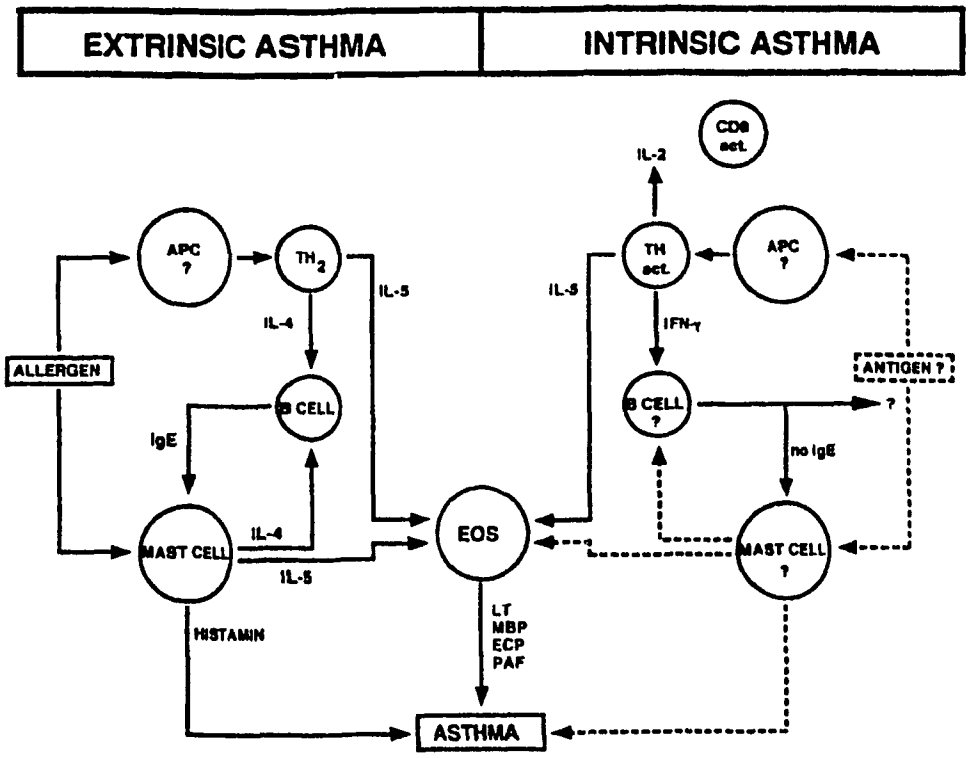

Fig. 1: Schematic representation of the postulated cellular mechanism of extrinsic and intrinsic asthma (Adopted from Walker, 1993).

1.3 Role of inflammatory cells in asthma

Various cells are associated with inflammatory events in asthma. Eosinophils and T-cells play a particularly significant role in asthma, especially in chronic allergic inflammation. These and the other inflammatory cells implicated in the pathogenesis of asthma, including mast cells, basophils, neutrophils and macrophages, will be discussed below.

1.3.1 Eosinophils

Eosinophils are non-dividing cells derived from bone marrow, which are recognised by their bi-lobed nuclei and granulated cytoplasm. Eosinophil accumulation plays a central role in the pathogenesis of helminthic parasitic disease (Weller, 1984), pulmonary eosinophilic syndrome (Enright et al., 1989) and bronchial asthma (Cutz et al., 1978).

There is increasing evidence to implicate eosinophil migration and localisation to airways as the main instigator of tissue damage in asthma. Post-mortem studies of asthmatics have demonstrated eosinophil infiltration of the bronchial mucosa (Filley et al., 1982; Cutz et al., 1978). An increased number of eosinophils has also been demonstrated in the peripheral blood of allergic and non-allergic asthmatic patients (Horn et al., 1975; Durham & Kay, 1985). The increased number of eosinophils correlated with the degree of bronchial hyperactivity (Durham & Kay, 1985) and with severity of the disease (Horn et al., 1975). In addition, eosinophils and their toxic granular products have been identified in the BAL fluid and

bronchial biopsies from asthmatics (Wardlaw et al., 1988; Azzawi et al., 1990). The importance of increased eosinophil numbers in the lungs of asthmatics was further supported by a strong correlation between symptom severity, airway responsiveness and disorder in lung function (Wardlaw et al., 1988; Bousquet et al., 1990).

Eosinophil-derived products have the capacity to produce many of the pathological and physiological features of asthma, such as airways constriction, airways hyperresponsiveness (Gundel et al., 1991) and desquamation of the epithelial cell layer (Motojima et al., 1989). Changes in the density distribution of peripheral blood eosinophils have been observed in individuals with atopic asthma who experienced LAR following inhalant allergen (Frick et al., 1989). Activated eosinophils were hypodense and showed enhanced cytotoxic activity compared to inactivated eosinophils.

Mature eosinophils contain many basic proteins including major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO) (Dvorak et al., 1991). These granule proteins are toxic to parasites, bacteria and host cells (Ackerman, 1993). A detailed analysis of the effect of MBP and ECP on the tracheal epithelium showed that they damage epithelial cells *in vitro* (Motojima et al., 1989; Gleich et al., 1979). Increased levels of MBP is present in the bronchial wall and mucus plugs of patients who have died from asthma attacks and this sites of MBP deposition coincided with the epithelium damage (Filley et al., 1982). Airway responsiveness has been correlated directly with peripheral eosinophil numbers and serum ECP concentration after bronchial allergen challenge (Rak et al., 1988).

Activated eosinophils also generate membrane derived-lipid mediators, including platelet activating factor (PAF) and leukotriene C_4 (LTC_4) (Lee et al., 1984; Shaw et al., 1985). These mediators may increase the allergic response by increasing vascular permeability (Wardlaw et al., 1986), and also by stimulating the adherence of eosinophils to endothelial cells (Kimani et al., 1988).

Eosinophils can also synthesise and elaborate a number of important proinflammatory cytokines which can elicit the cellular responses seen in asthma. These cytokines include TGF-β1 (Wong et al., 1991), IL-3 (Kita et al., 1991), IL-5 (Dubucquoi et al., 1994), TNF-α (Costa et al., 1993) and GM-CSF (Moqbel et al., 1991). Agents such as IL-5, regulated and normal T lymphocyte expressed and secreted (RANTES), and monocyte chemoattractant protein-3 (MCP-3) act as eosinophil chemoattractants and induce their recruitment into the airways (Resnick and Weller, 1993; Baggiolini and Dahinden, 1994). Recent studies have described a specific pathway for the recruitment of eosinophils from the blood into the site of allergic inflammation. This is accomplished by the specific adhesion molecule VCAM-1 which preferentially binds eosinophils via VLA-4, its complementary ligand on the eosinophil cell surface (Walsh et al., 1991a; Bochner et al., 1991). In guinea pigs, it has been shown that antibody against VLA-4 can inhibit antigen-induced bronchial hyperactivity, as well as eosinophil number in BAL fluid and bronchial tissues (Pretolani et al., 1994). These data suggest a close relationship between eosinophil infiltration into the airways and increased airway responsiveness.

Table 2: Products of Eosinophil and Their Functions in Asthma

Mediator	Function
Granule Products	
Major Basic Protein (MBP)	Cytotoxic
Eosinophil Cationic Protein (ECP)	Cytotoxic
Eosinophil-Derived Neurotoxin (EDN)	Cytotoxic
Eosinophil Peroxidase (EPO)	Cytotoxic
Activation Products	
Leukotriene C_4 (LTC_4)	Bronchoconstrictor
Platelet Activation Factor (PAF)	Bronchoconstrictor; chemotactic to eosinophils

These observations, taken together, suggest that recruited eosinophils in the airway may play a critical effector role in the pathogenesis of bronchial asthma by releasing various mediators.

1.3.2 Mast cells

Mast cells are derived from stem cells in the bone marrow. The hematopoietic cytokine IL-4 causes the differentiation and activation of mast cells (Hamaguchi et al., 1987). IgE-mediated activation of mast cells results in degranulation and release of mediators such as histamine, neutral proteases including tryptase, heparin, and lipid mediators including prostaglandin, PAF and LTC_4 (Galli, 1993). These mediators cause smooth muscle contraction, blood vessel dilation, increased capillary permeability, mucosal edema, cellular infiltration and mucus hyper-secretion in the airways (White and Kaliner, 1991). Current evidence suggests that mast cells may mediate the process underlying EAR. It has been reported that both histamine and mast cell tryptase are elevated in the BAL fluid during EAR after allergen challenge (Wenzel et al., 1988; Liu et al., 1991).

Recently it has been reported that mast cell activation results in the production of a number of cytokines including IL-4, IL-5, IL-6, and TNF-α (Bradding et al., 1993; Walsh et al., 1991b). Therefore, in addition to the mast cell's well-documented role in EAR, these mast cell cytokines could initiate the development of LAR by their ability to recruit and activate inflammatory cells such as eosinophils.

1.3.3 Basophils

Basophils are also derived from stem cells in the bone marrow. They are recognisable by their multilobed nucleus and large electron-dense granules. Basophils are involved in systemic and local allergic reactions to blood-borne antigens. The differentiation of basophils

appears to be similar to that of eosinophils and is T cell dependent. IL-3 induces selective differentiation and maturation of basophils (Valent et al., 1989). Upon activation, basophils release mediators including histamine and LTC_4 (Biscoff et al., 1990).

The mechanisms of basophil recruitment to human asthmatic airways is presently unclear although it has been suggested that cytokines, including IL-3, IL-5, GM-CSF and RANTES, are chemoattractants for basophils (Tanimoto et al., 1992). There is increasing evidence that basophils are involved in acute asthmatic attacks (Koshino et al., 1993) because of their recruitment to the lung during LAR (Guo et al., 1990; Liu et al., 1991). However, the events involved in the recruitment, activation and degranulation of basophils are not unique to atopic diseases because they also contribute to host defence against parasitic diseases (Askenase, 1980).

1.3.4 Macrophages

Macrophages are mononuclear cells derived from the bone marrow. They are the predominant cell type present in BAL fluid. Tissue macrophages process antigen and then present it to T lymphocytes (Johnston, 1988).

In asthmatic individuals, alveolar macrophages express a low affinity surface IgE receptor (FcεRII) (Joseph at al., 1983). However, the number of macrophages recovered from BAL fluid was similar between asthmatics and normal controls (Wardlaw et al., 1988; Metzger et al., 1986). Recent studies demonstrated that alveolar macrophages potently inhibit both mitogen- and antigen-stimulated T- and B-cell growth in normal human lung (Spiter and

Poulter, 1991) while the removal of alveolar macrophages resulted in an enhanced immune response in mice lungs (Thepen et al., 1989). Taken together, these reports indicate that macrophages may be a normalising control on the extent of inflammation. However, the role of macrophages in asthmatic inflammation needs yet to be elucidated.

1.3.5 Neutrophils

Mature neutrophils are granule-containing and phagocytic cells derived from the bone marrow. They are abundant in the peripheral blood. They are the first cells to be recruited to sites of inflammation. When neutrophils are activated, they release their granule contents such as PAF and LTB_4.

BAL obtained from asthmatic patients after inhalation challenge has demonstrated that neutrophils appear early in the asthmatic reaction (Metzger et al., 1986). However, the number of neutrophils in the bronchial biopsies of steady state, chronic asthmatics is similar to that in control subjects (Azzawi et al., 1990). Thus, although neutrophils play an essential role in inflammation, their precise function in the LAR and the extent of their involvement in the asthmatic response is unclear.

1.3.6 Lymphocytes

Lymphocytes are mononucleated cells produced from stem cells in the bone marrow. The immune response to foreign antigens elicited by lymphocytes can be broadly divided into two types: humoral and cell-mediated. The humoral response serves mainly to eliminate exogenous bacteria and bacterial products and is mediated by circulating antibodies produced

by B cells, which are themselves regulated by T lymphocytes. The cell-mediated response serves to recognise and eliminate altered self-cells, including virus-infected and malignant cells.

1.3.6.1 B Lymphocytes

B lymphocytes mature in the bone marrow and are activated in local lymphoid tissues. Activation by an antigen results in differentiation of B lymphocytes into antibody-secreting plasma cells and memory cells. Plasma cells secrete various immunoglobulin isotypes such as IgM, IgD, IgG_{1-4}, $IgA_{1,2}$ and IgE. These secreted antibodies function as multiple effector molecules of humoral immune responses. For example, the role of IgE in allergic inflammation is well established. *In vitro* studies have demonstrated that B lymphocyte production of IgE requires a cognate contact with allergen sensitised "helper" (CD4+) T cells and IL-4 (Romagnani, 1990).

Increased numbers of B cells have been reported in the bronchial biopsies of asthmatics (Laitenen et al., 1993). This suggests that B cells may produce IgE within the airways. Allergen binding to IgE molecules preattached to mast cells would then lead to the rapid release of mast cell mediators, and possibly the development of asthma (Laitenen et al., 1993).

1.3.6.2 T Lymphocytes

T lymphocytes mature in the thymus then migrate to local tissues. Primary antigen exposure causes T cells to differentiate from naive to memory T cells. Following secondary activation by the same antigen, T-cells become effector cells.

Increased numbers of T lymphocytes were observed in bronchial tissue samples from asthmatics (Jeffery et al., 1989). This lymphocytic infiltration has been correlated with the degree of eosinophil infiltration to the airways, suggesting that lymphocytes actively contribute to persistent airways inflammation (Azzawi et al., 1990).

The majority of T lymphocytes can be divided into two main subtypes based on their cell surface proteins, CD4 and CD8. T cells expressing the CD4 protein on their surface are known as helper T cells because they function as a "helper" in antibody production by B cells. CD4+ T cells only respond to antigens associated with class II MHC molecules. CD8+ T lymphocytes are cytotoxic, and associate with class I MHC molecules. CD4+ T cells play a central role in the pathogenesis of bronchial asthma because the cytokines elaborated by these cells regulate the production of IgE antibody by B cells and induce tissue eosinophilia. The former process is specifically regulated by IL-4 and IFN-γ whereas the latter by IL-5 (Kay, 1992).

Using specific antibodies against T lymphocyte subtypes, it has been possible to study changes in CD4+ and CD8+ T cells within the airways of asthmatics. An increase in CD4+ T cells was observed in BAL fluid 48 hours after allergen challenge in asthmatics who have previously shown LAR. This was accompanied with a decrease in the number of peripheral

blood CD4+ cells (Gerblich et al., 1991). This suggests that CD4+ T cells may be recruited from the intravascular space into BAL fluid after antigen provocation (Gerblich et al., 1984). Moreover, following allergen-induced EAR in asthmatic patients, an increase in CD8+ T lymphocytes was observed in BAL fluid and this was associated with the attenuation of LAR (Gonzalez et al., 1987).

These findings suggest that lymphocytes, and particularly activated CD4+ cells, may instigate the pathogenesis of asthma and allergic inflammation, not only by directing B cells to generate IgE, but also by secreting cytokines which act on effector cells to amplify the inflammatory response.

1.3.6.2.1 Th1 and Th2 subsets

Murine CD4+ T helper cell clones can be broadly designated as two distinct categories, type 1(Th1) and type 2 (Th2) helper T cells, based on the cytokine profile secreted in response to antigen (Mosmann et al., 1986; Cherwinski et al., 1987). Th1 cells predominantly elaborate IL-2 and IFN-γ while Th2 cells express IL-4 and IL-5 (Fiorentino et al., 1989). Both Th cell types produce IL-3, and GM-CSF. Recently, there has been convincing evidence to suggest that Th1-and Th2-like functional subsets exist in humans (Romagnani, 1992; Kay , 1994). A major interest of this T cell dichotomy is related to the different types of pathology associated with Th1 and Th2 responses. Th1 cells play a critical role in directing cell-mediated immune responses and clearance of infectious organisms (Cher and Mosmann, 1987). In contrast, the cytokines produced by Th2 cells are associated with allergy and are important for

humoral responses resulting in IgE production and eosinophilia (Mosmann and Coffman, 1989). Th1 and Th2 cells reciprocally inhibit each other by secreting IFN-γ and IL-4, respectively (Street and Mosmann, 1991). These two cell types may thereby balance each other in mediating certain types of antigen responses.

The local cytokine environment during the initial stages of T cell activation may determine the development of the appropriate Th cell phenotype. Th2 cells arise in the presence of IL-4 while Th1 type cells develop in the presence of IL-12 and IFN-γ (Romagnani, 1992).

Th2-type cells regulate allergic and asthmatic inflammation through their soluble cytokine products which control the survival, growth, differentiation and effector functions of tissue cells (Plaut, 1990; Kay, 1992). Increased numbers of BAL cells expressing mRNA for IL-3, IL-4, IL-5 and GM-CSF have been detected from the airways of atopic asthmatic subjects; these numbers have correlated with eosinophil infiltration and disease severity (Robinson et al., 1992).

1.4 Interleukin-5

IL-5 is the most important cytokine implicated in the selective accumulation, activation and maintenance of airway eosinophilia in asthma (Hamid et al., 1991; Ying et al., 1995). IL-5 has a molecular weight of 18 KDa as a monomer. However, it exists functionally as an antiparallel homodimeric glycoprotein linked by two disulphide bonds (Minamitake et al., 1990). The tertiary crystalline structure of IL-5 shows a novel two domain structure

containing four α-helices (Milburn et al., 1993). Each bundle of four helices consists of three strands from one monomer and one strand from the other. T cells were initially believed to be the main source of IL-5 (Schimple and Wecker, 1972), but recent studies in humans have demonstrated that other cell types, such as eosinophils and mast cells, are also sources of IL-5 (Broide et al., 1992; Bradding et al., 1993).

The human IL-5 gene was cloned by several groups (Kinashi et al., 1986; Azuma et al., 1986; Campbell et al., 1987). Located on chromosome 5, the human IL-5 gene codes a sequence containing 4 exons which encode a peptide of 124 amino acids (van Leeuwen et al., 1989). Interestingly, the genes for the inflammatory cytokines of the IL-4 family, including IL-3, IL-4, IL-13, and GM-CSF, are also clustered on human chromosome 5 (Boulay and Paul, 1992a; 1992b). IL-5 shares similar exon structures, protein primary sequences, receptors, cellular sources, and functional activities with these cytokines from the IL-4 family. Thus, they are evolutionary related cytokines. In mice, the genes for these cytokines are closely linked on chromosome 11 (Boulay and Paul, 1993).

Human and murine IL-5 exhibit 77% identity at the nucleic acid level and 70% identity at the amino acid level (Azuma et al., 1986). IL-5 is responsible for many physiological functions. IL-5 is important in the terminal differentiation of committed eosinophil precursors (Clutterbuck et al., 1989; Yamaguchi 1988a). It activates mature eosinophils and prolongs their survival in culture (Yamaguchi et al., 1988b), as well as selectively enhances eosinophil degranulation, antibody-dependent cytotoxicity and adhesion to vascular endothelium (Lopez et al., 1988; Fujisawa et al., 1990; Walsh et al., 1990). IL-5 also primes basophils, leading to

increased histamine granulation and LTC_4 generation (Bischoff et al., 1990), and increases synthesis of IgM, IgA, IgE by and expression of surface marker CD23 on B cells treated with IL-4 (Pene et al., 1988; Purkerson and Isakson, 1992).

Table 3: Interleukin -5 and Local Inflammation

Cytokine	Cellular Source	Cellular Target	Effect
IL-5	T_H cells (T_H2 subset) Mast cells Eosinophils	Eosinophils B cells T cells Basophils	Induces eosinophil growth differentiation, chemotaxis and activation. Primes Basophils. Acts with IL-4 to stimulate IgE production in B cells. Induces B-cell proliferation and differentiation.

1.4.1 Interleukin-5 associated with bronchial asthma

There are a number of studies demonstrating the role of T lymphocyte derived-cytokines, particularly IL-5, in the control of eosinophil function in asthma. The increased expression of IL-5 message by CD+ T lymphocytes has been demonstrated in the bronchial biopsies from asthmatics (Hamid et al., 1991; Ying et al., 1995). A close correlation was found between increased IL-5 mRNA expression by activated lymphocytes and eosinophil infiltration at these sites, indicating that IL-5 has an important role in regulating eosinophil

function in airway inflammation of asthmatics. Increased expression of IL-5 mRNA has also been observed in BAL T lymphocytes (Robinson et al., 1992) and peripheral blood CD+ T cells (Corrigan et al., 1995). The increased expression of IL-5 mRNA was correlated closely with asthma severity and airway responsiveness (Robinson et al., 1993a). Furthermore, bronchial biopsies obtained from asthmatic patients following allergen inhalation contained an increased number of cells expressing IL-5 mRNA (Bentley et al., 1993). Increased IL-5 protein levels have been observed in serum, BAL, peripheral blood T cell supernatants from patients with bronchial asthma (Walker et al., 1991, 1992; Motojima et al 1993). Significant correlations have been observed between increased IL-5 protein and the number of eosinophils in peripheral blood and BAL fluid in bronchial asthma (Walker et al., 1992). IL-5 recovered from BAL was associated with eosinophil survival, recruitment, degranulation, and lung injury in the antigen-induced pulmonary late phase reaction (Ohnishi et al., 1993a; 1993b).

Clinical symptoms of asthma are often successfully treated with corticosteroids. Studies of the effects of corticosteroids in asthma support a role of IL-5 in the pathogenesis of this disease. Glucocorticoid treatment resulted in the reduction of IL-5 levels and eosinophil number in the peripheral blood of patients with asthma (Corrigan et al., 1995). These investigators suggested that IL-5 secreted by activated T lymphocytes regulates eosinophilia and that treatment with glucocorticoid reduces T cell activation and thereby IL-5 production and eosinophilia. Recent studies indicated that the profiles of cytokine mRNA detected in BAL and lung tissue from asthmatics were altered by prednisolone therapy; there was a significant decrease in activated T cells expressing IL-5 mRNA, but an increase in IFN-γ mRNA positive

cells (Robinson et al., 1993b; Bentley et al., 1996). Furthermore IL-5 mRNA positive cells in steroid-sensitive asthma were decreased but not in steroid-resistant asthma (Leung et al., 1995).

Further evidence implicating a role for IL-5 in asthma derives from animal studies. The ovalbumin sensitised guinea pig is a well-established experimental model to study allergic asthma. Guinea pigs are first sensitised by injection or aerosol inhalation of ovalbumin. Subsequent challenge with ovalbumin several weeks later induces EAR and LAR, eosinophilia, and hyperresponsiveness in the airways. In sensitised guinea pigs pre-treated with IL-5 antibody, infiltration of eosinophils into the airways is reduced following ovalbumin challenge (Chand et al., 1992; Gulbenkian et al., 1992). Furthermore, anti-IL-5 mAb therapy inhibited the increase in eosinophil levels in BAL as well as bronchial hyperresponsiveness in chronically challenged, ovalbumin- sensitised guinea pigs (van Oosterhout et al., 1993a). However, anti-IL-5 treatment did not alter neutrophil counts in these animals. Treatment of sensitised guinea pigs with anti-IL-5mAb markedly blocked the development of hyperractivity to histamine following ovalbumin inhalation (van Oosterhout et al., 1993b). These data collectively argue that IL-5 is important in the recruitment of eosinophils into the airways and in the development of hyperreactivity in this animal model.

In recent years, it has been widely recognised that eosinophils are the most important inflammatory cells in asthma and that the IL-5 might be the main mediator in regulating the eosinophil function in asthma.

1.4.2 IL-5 in other diseases

Other diseases are also linked to the expression of IL-5 and the development of eosinophilia. Allergic states such as cutaneous late-phase reactions, atopic dermatitis and allergic rhinitis are all associated with IgE-mediated degranulation of mast cells and cellular accumulation of inflammatory cells, particularly in activated T lymphocytes and eosinophils. Allergic inflammation has been described in cutaneous late-phase reactions in which chronic infiltration of the dermis, with increased numbers of lymphocytes and eosinophils, has been observed in skin biopsies of atopic patients following allergen provocation (Frew & Kay, 1988). Increased expression of IL-5 mRNA has been demonstrated in allergen-induced late-phase cutaneous reactions without changes in IFN-γ or IL-2 mRNA (Kay et al., 1991). In addition, atopic dermatitis is characterised by pruritus, high levels of serum IgE and increased numbers of eosinophils in the blood. The maintenance of chronic atopic dermatitis has been predominantly associated with an increased expression of IL-5 message and accumulation of eosinophils at these sites (Hamid et al., 1994). Furthermore, increased numbers of eosinophils, lymphocytes and mast cells have been observed in nasal biopsies of patients with allergic rhinitis during allergy season. Outside the allergy season, increased expression of IL-5 mRNA has been demonstrated in the nasal mucosa from atopic allergic rhinitis patients after local antigen challenge. This increase in IL-5 message closely correlated with the infiltration of activated eosinophils (Durham et al., 1992).

Other non-allergic diseases associated with eosinophil accumulation include helminth-induced inflammation, hypereosinophilic syndrome, and cancer. Filarial parasite infection in

humans induces eosinophilia and increased levels of IL-5 mRNA and protein in the blood (Limaye et al., 1990). The expression of IL-5 message in the spleen of mice infected with *Taxocara canis* is closely linked with eosinophilia (Yamaguchi et al., 1990). However, when parasite-infected mice were treated with anti-IL-5 mAb, they have failed to develop eosinophilia (Coffman et., 1989). These reports suggest that IL-5 is essential in supporting eosinophilia in a parasite-induced eosinophilic response.

In patients with hypereosinophilic syndrome, there is a predominance of hypodense (low-density) eosinophils. These low density cells have fewer cytoplasmic granules (Winquist et al., 1982), have phagocytic and cytotoxic capacities for helminthic targets (Prin et al., 1983) as well as generate lipid mediators, such as LTC_4, after physiological stimulation (Shaw et al., 1985). Such characteristics indicate that the hypodense eosinophils are activated, raising the possibility that IL-5 is responsible for the activation of these eosinophils as well.

A positive correlation between eosinophil infiltration and survival rates in individuals with stomach or colon cancer has been demonstrated by histological studies (Iwasaki et al., 1986; Pretlow et al., 1983). The use of IL-2 as tumour therapy is associated with the development of eosinophilia by inducing IL-5 production (Macdonald et al., 1990). Thus, eosinophils, whose production is mediated by IL-5, correlate with a positive prognosis in cancer patients.

1.4.3 IL-5 and eosinophils

There are various *in vitro* studies to indicate that IL-5 is a major, possibly the most important, cytokine involved in the regulation of eosinophil function.

IL-5 stimulates eosinophil colony formation. Studies of human bone marrow established that recombinant human IL-5, IL-3 and GM-CSF are all capable of stimulating the development and differentiation of eosinophils. However, only IL-5 was selective for the eosinophil lineage since GM-CSF and IL-3 also promote neutrophil and macrophage production (Clutterbuck et al., 1989).

IL-5 activates eosinophils. When eosinophils were incubated with recombinant IL-5, changes in cell morphology were observed, including irregular shape and membrane ruffling. In addition to these morphological changes, eosinophils stimulated by IL-5 showed greater cytotoxocity against tumour cells (Lopez et al., 1988). IL-5 can also stimulate the production of lipid derived mediators such as LTC_4 in eosinophils (Takafuji et al., 1991).

IL-5 is a specific chemoattractant for eosinophils. This was shown by an *in vitro* migration assay (Wang et al., 1989). IL-5 was subsequently shown to be a weaker, yet more selective, eosinophil chemoattractant than other chemotactic agents such as RANTES and MCP-2 (Kameyoshi et al., 1992). In addition, IL-5 can enhance eosinophil chemotactic activity to other non-specific chemotactic agonists such as LTB_4 and PAF (Schmi et al., 1992). Moreover, IL-5 selectively upregulated adhesion of human eosinophils via its VLA-4 integrin to the endothelial cell ligand VCAM-1 *in vitro* (Walsh et al., 1990; 1991). Antibodies against VLA-4 abolished the adhesion. Together, these results indicate that *in vivo,* these integrins may be associated with selective priming of eosinophils locomation by IL-5.

IL-5 is important in prolonging the survival of eosinophils *in vitro.* It was shown that murine IL-5 (8 Units/ml) supported eosinophil survival in culture for up to two weeks, while higher concentrations of IL-3 (100 U/ml) and GM-CSF (100 U/ml) supported eosinophils for

four and eight days, respectively (Yamaguchi et al., 1988b). In addition, treatment with serum from individuals with asthma or hypereosinophilic syndrome enhanced eosinophil survival in culture; this activity was partly abolished in the presence of antibody against IL-5 (Owen et al., 1989). It is therefore likely that serum IL-5, which is high in these individuals, was responsible for the enhanced eosinophil survival. IL-5's role as an eosinophil survival factor may be due to its ability to delay apoptosis (Yamaguchi et al., 1991; Stern et al., 1992). This likely involves the IL-5 receptor α-chain because overexpression of IL-5 receptor α-chain (αIL-5R) mRNA suppressed apoptosis in an IL-5 dependent cell line derived from human erythroleukemic cells (Yen et al., 1995).

IL-5 alone can induce production of specific eosinophilia. *In vivo,* IL-5 transgenic mice were demonstrated to have profound natural eosinophilia in their blood and spleen and to contain a large number of eosinophil precursors in their bone marrow (Dent et al., 1990).

1.5 Cytokine Receptors

Cytokines are secreted cell-regulatory molecules. They produce cellular responses such as growth and differentiation through receptor-specific activation of intracellular signal transduction pathways which regulate gene expression (Nicola et al., 1989). In general, cytokine receptors are in low abundance on the surface of the target cells, 100-1,000 receptors per cell, hindering biochemical characterisation in detail (Gillis, 1991).

Cytokine receptors can exist in both soluble and membrane-anchored isoforms. Soluble cytokine receptors that have been described, include IL-2R, IL-4R, IL-5R, IL-7R, and

TNFR-α (Fernandez-Botran, 1991; Tavernier et al., 1992; Devos et al., 1993). The soluble receptors have similar binding affinities as the membrane anchored form , yet lack the transmembrane and intracytoplasmic domains. The function of soluble cytokine receptors is not clear; however, they may act as physiological modulators of cytokine action. For example, binding of an cytokine to a soluble receptor in the biological fluids may hinder its binding to a functional transmembrane receptor (Fernandez-Botran, 1991).

Cytokine receptors are grouped into at least four families based on their structural similarities. First, the hematopoietin/cytokine receptor family is the largest of the cytokine receptor gene families with conserved cysteine residues and Trp-Ser-Xaa-Trp-Ser motif in the extracellular domain (Bazan, 1990). This family include the receptors for IL-2R (β and γ chains), IL-3R (α and β chains), IL-4R, IL-5R (α and β chains), IL-6Rgp130, IL-7R and GM-CSFR (α and β chains). Second, the immunoglobulin (Ig) -receptor family recognised by conserved specifically spaced cysteine residues, three extracellular Ig-like domains linked by intra-chain disulphide bonds, a short transmembrane domain and cytoplasmic domains of variable lengths (Foxwell et al., 1992). The receptors are included in this family are IL-1R and M-CSFR. Third, growth factor receptor family is characterised by rich cysteine-rich domain on the extracellular domain and tyrosine kinase activity on the cytoplasmic domain. They include receptors for epidermal growth factor, prostaglandin growth factor and insulin (Yarden and Ullrich, 1988). Fourth, TNF-receptor family contains conserved six cysteine residues in the extracellular domain in addition to cytoplasmic sequence similarities. This family includes both

TNF receptors and cell surface antigens CD30 and CD40, a nerve growth factor receptor (Gillis, 1991).

The activity of the cytokine on target cells is directly dependent on the initial binding to specific cell-surface receptor thus providing the physical link between cytokine and its function in disease states.

1.6 Interleukin-5 Receptors

The *in vivo* localisation of αIL-5 receptor expression in asthmatic airways would support a link between increased IL-5 levels and preferential eosinophil infiltration and activation at these sites. The human IL-5 receptor complex is a heterodimer consisting of an α subunit (αIL-5Rm) and a β-subunit (Murata et al., 1992; Tavernier et al., 1991). The observed molecular mass of the αIL-5Rm and the β-subunit are 60 KDa and 130 KDa, respectively (Plaetinck et al., 1990; Murata et al., 1992; Miyajima et al., 1993). Human IL-5 receptor expression is limited to eosinophils and basophils (Migita et al., 1991; Lopez et al., 1990). The human membrane-anchored IL-5R α-chain can bind hIL-5 with low affinity by itself. In contrast, the β-chain alone cannot bind IL-5 with detectable affinity. The combination of membrane-anchored α with β forms a high affinity interaction with IL-5 (Tavernier et al., 1991). The α subunit is exclusive for the IL-5 receptor but the β-subunit is shared between receptors for human IL-3, GM-CSF, and IL-5 (Tavernier et al., 1991; Lopez et al., 1991). These three cytokines belong to the class I cytokine family based on their structural motifs (Boulay and Paul, 1993).

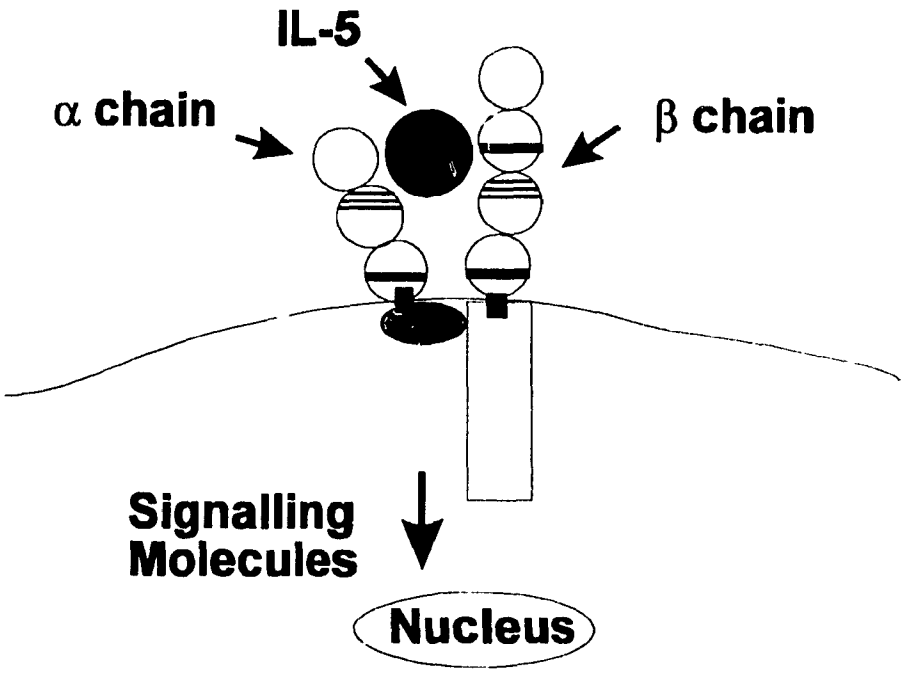

Fig. 2 : Structure of the heterodimeric human Interleukin-5 Receptor Complex: The α chain has three conserved extracellular submodules and the β chain has four extracellular submodules. Conserved cysteines are shown as fine lines and the sequences of the Trp-Ser-X-Trp-Ser motifs are shown by thick lines. The hatched box and open box represent the cytoplasmic tails of the receptor α and β-subunits, respectively. The proposed binding site for IL-5 is indicated.

The gene for the human αIL-5R subunit is located at chromosome 3 (Isobe et al., 1992; Tuypens et al., 1992), whereas the gene for the β chain is at chromosome 22 (Shen et al., 1992).

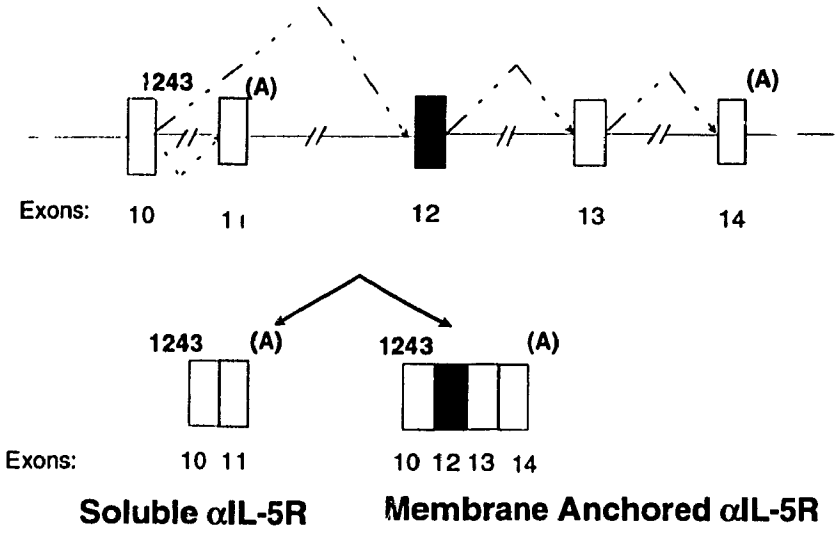

Fig. 3 : Alternative processing of human αIL5R genomic regions determining the soluble and membrane bound isoforms. Boxes represent exons while intervening lines represent introns. The hatched box (exon 11) encodes the 3' terminus of the soluble α chain mRNA and the solid bar (exon 12) is unique to the α chain of the membrane-bound isoform. Dashed arrow below, and dashed-dotted arrows above represent splicing patterns of the αIL5R producing soluble and membrane-anchored isoforms, respectively. (A) represents polyadenylation signals. The site of the splice donor on human αIL5R is indicated by the number (1243) (Adapted from Tavernier et al., 1992).

αIL-5R isoforms are generated by splicing of the single αIL-5R gene which contains 14 exons (Fig. 3) (Tuypens et al., 1992; Tavernier et al., 1992). Exon 11 encodes four amino acids followed by a stop codon and a polyadenylation site at the 3' terminus. Exon 12 encodes a transmembrane domain, while exons 13 and 14 encode the cytoplasmic tail. A membrane anchored isoform (αIL-5Rm) is formed by alternative splicing out exon 11, whereas a soluble isoform (αIL-5Rs) is generated by normal splicing in exon 11 (Tuypens et al., 1992; Tavernier et al., 1992).

The soluble αIL-5R isoform could have a regulatory function. This αIL-5Rs isoform binds human IL-5 with equally high affinity as the membrane-anchored IL-5R complex (Devos et al., 1993). IL-5 bound to αIL-5Rs prohibits the possible interaction of αIL-5Rs with the membrane-bound β-subunit. The αIL-5Rs variant therefore has antagonistic properties and can compete for ligand with the membrane-anchored IL-5R present on eosinophils (Tavernier et al., 1991). The recombinant αIL-5Rs isoform interacts with the IL-5 homodimer with a molar stoichiometry of a 1:1 ratio (Devos et al., 1993). Aside from *in vitro* studies in which the αIL-5Rs has been demonstrated to bind IL-5 and to inhibit IL-5 induced eosinophil proliferation (Tavernier et al., 1991; Devos et al., 1993), the *in vivo* function of the αIL-5Rs has yet to be elucidated.

1.6.1 Structural and functional characteristics of IL-5R

The membrane-anchored human αIL5R subunit cDNA encodes a protein of 420 amino acids, including an amino-terminal signal peptide of 20 amino acids, a glycosylated extracellular domain of 324 amino acids, a single transmembrane spanning segment of 21 amino acids and a cytoplasmic tail of 55 amino acids (Murata et al., 1992). The β subunit is 897 amino acids long with a transmembrane spanning segment of 27 amino acids and a 350 amino acid long cytoplasmic domain. The 200 amino acids proximal to the transmembrane region of the αIL-5Rm have common structural features with the cytokine/haemopoietin receptor superfamily (Bazan, 1990). This homologous region is characterised by a Trp-Ser-X-Trp-Ser motif and by several conserved short sequence elements, including four cysteine residues in the amino terminal half of this region which are predicted to form two disulphide bridges (Bazan, 1990). The integrity of the Trp-Ser-X-Trp-Ser motif in the receptor is required for the interaction with its ligand (Miyazaki et al., 1991). This homologous region is also composed of two fibronectin type III modules. Each fibronectin type III module is approximately 100 amino acids long and is comprised of seven β- strands positioned antiparellel to each other, forming a "barrel-like" shape. A trough formed between two "barrel-like" modules is believed to function as the ligand binding pocket (Bazan, 1990).

When a stretch of charged amino acids (Asp-55, Asp-56, Tyr-57, and Glu-58) in the amino terminus of mouse/human chimera molecules of αIL-5R were mutated to alanine, binding of IL-5 was affected (Cornelis et al., 1995a), indicating that these amino acids in αIL-5R are responsible for the specific binding of human IL-5. In addition, modification of the

cysteine residue (Cys66) by isothiazolone compound in the amino terminal domain of αIL-5R resulted in a receptor with reduced IL-5 binding affinity. Cys66 in the amino-terminus of the receptor may thus be critical for IL-5 binding (Devos et al., 1994).

1.6.2 IL5R mediated signalling mechanisms

IL-5R mediated signalling requires the cytoplasmic domains of both the α- and the β-subunits (Takaki et al., 1993; Sakamaki et al., 1992). When the β-chain is cotransfected with the αIL-5R, IL-5 crosslinks the two subunits together (Takaki et al., 1993; Sakamaki et al., 1992), indicating that dimerization of the α- and β-chains is an essential step for receptor activation and signal transduction.

Although neither the αIL-5R nor β subunit contain consensus sequences for intrinsic kinase activity (Mui et al., 1994), the binding of IL-5 to its receptor complex is associated with the involvement of tyrosine kinases in the first step of the signalling cascade and with the activation of several intracellular proteins (Sakamaki et al., 1992). These proteins include Lyn, Ras, Raf-1, MEK and microtubule associated protein (MAP) kinase (Pazdrak et al., 1995). Lyn is the member of the family of Src kinases which associates with the IL5R β- chain in unstimulated eosinophils; activation of Lyn results in the phosporylation of Ras. The other proteins are sequentially phosphorylated in turn to cause transcriptional regulation within eosinophils.

Furthermore, proline rich sequences in the membrane proximal region of the cytoplasmic domain of the α chain has been demonstrated to be responsible for the

phosphorylation of JAK2 bound to the β subunit, a cytoplasmic tyrosine kinase, upon the interaction of IL-5 with the IL5R (Cornelis et al., 1995b). JAK2 is a member of the Janus family of tyrosine kinases (JAKs). JAKs transduce signals via members of a novel family of transcriptional factors named signal transducers and activators of transcription (STATs) (Ihle et al., 1995). In a resting cell, STAT proteins are inactive in the cytoplasm. Following tyrosine phosphorylation by JAK, they translocate to the nucleus where they initiate gene transcription. It has been demonstrated that IL-5R activation results in the phosphorylation and activation of JAK2 tyrosine kinase and STAT1α in human eosinophils (Van der Braggen et al., 1995), providing another mechanism by which IL-5 may induce biological responses.

A functional promoter region upstream of the transcriptional start site of the αIL5R has been mapped to a position 15 bp upstream of the N-terminus. It is activated upon binding of a nuclear factor(s) in eosinophil-inducible myeloid leukemic cell lines *in vitro* (Sun et al., 1995).

Information is presently limited regarding the transcriptional mechanisms critical to the expression and regulation of IL5R. However, the identification of transcriptional factors and regulatory elements involved in IL-5R signalling may be a potential target for therapeutic intervention.

1.6.3 Species Differences of IL5R

The mouse IL-5 receptor has similarities with the human IL-5R but some differences do exist. In addition to the selective expression of IL-5R on eosinophils and basophils as in humans (Migita et al., 1991; Denburg et al., 1991), the IL-5R is also expressed on B cells in

mice (Imamura et al., 1994). It is composed of two distinct binding components, an unique 60-KDa membrane-bound αIL-5R chain with a 130-KDa β chain common to other cytokine receptors (Devos et al., 1991; Takaki et al., 1991). The membrane-bound mouse αIL-5R is composed of 415 amino acids and retains features characteristic of the cytokine/haemopoietin receptor family. The soluble isoform of mouse IL5Rα cDNA have been cloned (Takaki et al., 1990). The mouse αIL-5R cDNA has considerable homology with the coding sequence of the human αIL-5R, being 70% identical at the amino acid level (Murata et al., 1992). The human and mouse β chains are 55% identical at the amino acid level (Hayashida et al., 1990; Devos et al., 1991). The murine αIL-5R and β chains are located on chromosome 6 and 15, respectively (Imamura et al., 1994; Gorman et al., 1992).

1.6.4 IL-5R antagonists

Mutation of Glu-13 in human IL-5 reduced cell proliferation to 0.1% of the wild-type activity by decreasing receptor activation without affecting IL-5 binding to human αIL-5R in a human erythroleukemia cell line (Tavernier et al., 1995). This IL-5 mutant then has the potential to be a competitive antagonist of IL-5 because it binds equally well to the αIL-5R chain but has lost its ability to activate the receptor complex.

In the peritoneal cavity of IL-5 transgenic mice, the IL-5R was expressed on mature eosinophils (Hitoshi et al., 1991). When these mice were injected with anti murine-IL-5R mAb, a reduction in peripheral blood eosinophils to normal levels was observed within 5 days

(Hitoshi et al., 1991). Administration of anti-IL-5R mAb was thus able to antagonise IL-5 *in vivo.*

Therefore, receptor antagonists that bind specifically to IL-5R can compete with the biologically active IL-5 for binding to the same membrane receptor and may have a clinical significance since they can modulate IL-5 biological function.

2.0 OBJECTIVES OF THE STUDY

To better understand the action of IL-5 in asthma, it is essential to identify the site of IL-5 receptor synthesis and expression. Although receptor sites for IL-5 have been identified in various cells *in vitro,* the site of expression of the αIL-5R and the site of its synthesis in bronchial tissue of asthmatics is not clear.

The objectives of this study were :

1) To localise the expression of αIL-5R isoforms mRNA in airway biopsies from atopic and non-atopic asthmatics and compare them with those of atopic and non-atopic controls.

2) To determine the main cell type(s) expressing the αIL-5R in asthmatic biopsies.

3) To correlate the expression of αIL-5R isoform mRNA with clinical manifestations, airway obstruction (FEV_1) and bronchial hyperresponsiveness (PC_{20}) in asthma.

3.0 BACKGROUND ON THE TECHNIQUES UTILISED

Various methods were employed to study αIL-5R mRNA. Reverse Transcription Polymerase Chain Reaction (RT-PCR) and *in situ* hybridisation (ISH) were used to investigate the expression of αIL-5R mRNA while simultaneous *in situ* hybridisation and immunocytochemistry (ICC) were used to characterise the cell type expressing αIL-5R mRNA.

3.1 Reverse Transcription Polymerase Chain Reaction

This technique was used to confirm the presence of IL-5R mRNA in the bronchial biopsies from the subjects studied before employing the more rigorous technique of *in situ* hybridisation. mRNA can be selectively amplified by a reverse transcription polymerase chain reaction in the presence of specific primers which enables the detection of minute amounts of mRNA from tissues (Fig. 4) (Watson et al., 1989). In order to overcome variability in RNA purification, results are usually compared with the expression of a "housekeeping" gene such as β-actin which is constitutively expressed. Nonetheless, unlike ISH, this methodology does not provide information about the localisation of the gene in intact cells nor about the morphology of the cells expressing the mRNA of interest.

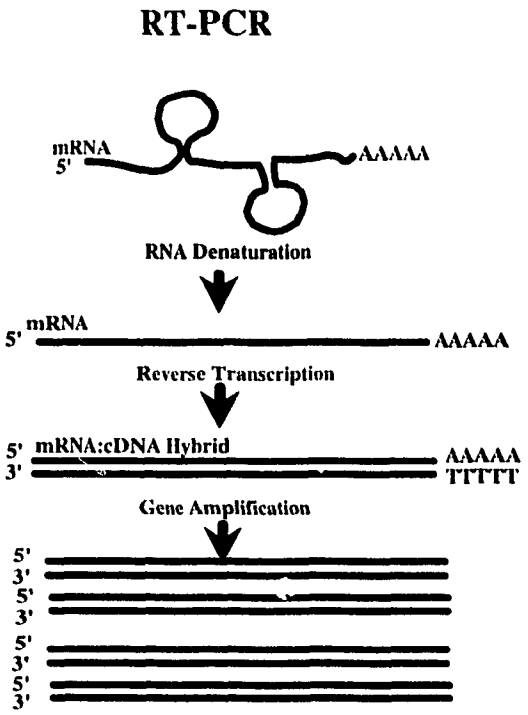

Fig. 4 : Schematic illustration of RT-PCR.

3.2 In Situ Hybridisation

In situ hybridisation is a technique used to determine the cellular localisation of specific gene expression. It uses a labelled probe to specifically bind complementary nucleotides in tissue sections. The two intracellular nucleic acid forms, DNA and RNA, are respectively located in the nucleus and the cytoplasm so the methodologies used to identify the location of these molecules are distinct. ISH was originally used to identify specific DNA targets (Gall & Pardue, 1969) but recently, ISH has been widely used to

detect specific intracellular mRNA populations. The cellular localisation of mRNA is of value for investigating specific gene expression and suggests the possible synthesis and function of proteins of interest. Several probes are available to identify mRNA, including oligonucleotides, single and double stranded DNA, and single stranded RNA. Recently, the identification of cytokine mRNA target cells in different tissue systems using single stranded RNA probes has been more widely used (Kay et al., 1991; Hamid et al., 1991; Robinson et al., 1992). This type of probe is preferred over the other types because a defined probe size may be synthesised, the thermal stability of RNA hybrids is higher, and the removal of unbound probes by posthybridisation with RNAse treatment while retaining the specific hybrids is possible (Cox et al., 1984). Autoradiography reveals the tissue distribution of specific mRNA, and therefore the site of the gene expression.

The technique of *in situ* hybridisation therefore provides a sensitive and reliable methodology to identify intracellular mRNA while retaining the morphology of the cell. Thus, ISH was chosen as the main technique for this thesis work.

3.3 Immunocytochemistry

Immunocytochemistry was designed to localise antigens in cryostat tissue sections by employing specific antigen-antibody reactions. Several methods are available for immunocytochemistry including direct and indirect methods. This thesis utilised an indirect ICC method called alkaline phosphatase-anti-alkaline phosphatase (APAAP) staining (Cordell et al., 1984). APAAP is an enzyme/anti-enzyme complex used as the label detection reagent. The cryostat tissue sections are first incubated with unlabelled

primary monoclonal antibodies and then with unlabelled secondary antibody. Specifically bound antibody is then visualised by treatment with APAAP complex and substrate. This method is very valuable since it is sensitive in detecting low amounts of antigen.

3.4 Simultaneous in situ hybridisation and immunocytochemistry

Combining immunocytochemistry to detect cellular antigens with subsequent *in situ* hybridisation to localise mRNA provides a powerful tool for determining the relationship between mRNA expression and the protein expression, allowing determination of the phenotype of mRNA positive cells. This procedure requires compatibility between proteolytic digestion and denaturation conditions for both antigens and nucleic acids as well as adequate colour contrast between the substrate products.

4.0 MATERIALS AND METHODS

4.1 Subjects and Study design

Asthmatic and non-asthmatic controls studied were recruited from the Allergy Clinic at the Royal Brompton National Heart and Lung Hospital, London, England. Endoscopic biopsy specimens from asthmatic and non-asthmatic controls were obtained by Dr. M. Humbert using fiberoptic bronchoscopy. These biopsies were then transferrd in dry-ice to Meakins-Christie Laboratories, McGill University, Montreal, Canada, in order to be utilised for this thesis work. The subjects investigated were comprised of 19 stable asthmatics (atopic asthmatics n=9 and intrinsic asthmatics n=10) and 20 controls (atopic controls n=10 and non-atopic normal controls n=10). Clinical characteristics of the subjects are shown in Table 5. The diagnosis of asthma was as previously described (Wardlaw et al., 1988). In brief, all the patients showed typical clinical symptoms: documented airway obstruction reversibility and increased airway responsiveness to methacholine (PC_{20} < 8 mg/ml, where PC_{20} is the provocation concentration of inhaled methacholine that caused a 20 % decrease in FEV_1). Atopic status was determined by skin prick testing to a range of ten common environmental aeroallergens. A skin response with a wheal 3 mm or greater in diameter to at least one allergen was considered positive for atopy. Serum IgE levels in atopic asthmatics ranged from 57 to 374 U/L and in non-atopic asthmatics ranged from 17 to 108 U/L. None of the asthmatics had taken inhaled or oral corticosteroids or other prophylactic treatment in the preceding 3 months. The nonatopic healthy control subjects were recruited from the local population. All subjects (asthmatics and

controls) were non-smokers. None of these subjects had evidence of any other pulmonary disease and none had suffered from a respiratory tract infection during the two months preceding the study. The study was performed with written informed consent and with the approval of the Royal Brompton Hospital Ethics Committee. For each subject, baseline spirometry and forced expiratory volume in one second (FEV_1) were recorded and a methacholine provocation test was performed. Bronchial biopsies were obtained from the subsegmental airways as described (Azzawi et al., 1990). Nebulized salbutamol was given to all subjects (asthmatics and controls) before the bronchoscopy procedure.

4.2 Processing of Biopsies

The bronchial biopsies were either frozen in OCT compound (Miles) or fixed for two hours in 4% paraformaldehyde/PBS solution, which maintains morphological integrity while allowing efficient hybridisation. Fixed biopsies were then washed in 15% sucrose/PBS three times. Cryostat blocks of frozen or fixed biopsies were prepared by embedding in OCT compound followed by immersion in isopentane cooled in liquid nitrogen and stored at -70°C.

4.3 Total RNA Isolation

Total RNA was isolated from frozen lung tissues using TRIzol reagent (Gibco-BRL). Every tissue sample was treated with 1 ml of TRIzol then homogenized with a 10 ml syringe with a 21 gauge $1^1/_2$ needle to increase cell lysis by aspiration and forceful expulsion of the fluid. Samples were then incubated at room temperature for 5 minutes, treated with 0.2 ml of

chloroform, vortexed and centrifuged at 12,000 × g for 15 mins at 4°C. The RNA layer was collected, precipitated with 0.5 ml of isopropyl alcohol, centrifuged at 12,000 x g, washed in 75% ethanol, air dried and dissolved in diethylpyrocarbonate (DEPC) treated H_2O. A spectrophotometric A260/280 ratio of >1.8 was considered to be a pure RNA preparation.

4.4 Reverse Transcription-Polymerase Chain Reaction

Reverse transcription was performed on the isolated RNA samples. 1 µg of Oligo dT (Gibco-BRL) was added to 2 µg of total RNA to make a total volume of 10 µl in DEPC treated H_2O. The mixture was heated for 3 min at 68 °C, then the samples were left on ice for 10 min. The following was added to the above mixture for a final volume of 20 µl: 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 0.2 mM of dGTP, dATP, dTTP, and dCTP, 1 U of Ribonuclease inhibitor (RNasin) (Promega), and 200 U of Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (Gibco- BRL). The reaction mixture was then incubated for 1 hour at 37 °C.

The reverse transcribed products of the RNA samples were amplified using specific primers for αIL-5Rm and αIL-5Rs under non-saturating conditions (Table 4). β-actin was used as a "housekeeping" gene, which is constitutively expressed. The optimal concentration of magnesium was determined to be 2 mM for each set of primers. 4 µl of the reverse transcribed products were amplified using specific primer (Sheldon Biotechnology Center, Montreal) in a master mix containing the following: 25 pmol of each sense and antisense primers, 0.2 mM of dATP, dCTP, dGTP, and dTTP (Gibco-BRL), PCR buffer (20 mM Tris-HCl (pH 8.4), 50 mM

KCl) (Gibco- BRL), 2 mM $MgCl_2$ (Gibco- BRL) and 1 U of Taq polymerase (Pharmacia). The PCR was performed in an automatic thermal cycler (MJ Research) as follows for 30 cycles: denaturation at 94°C for 1 min, annealing at 52°C for $1^1/_2$ min, and extension at 72°C for 2 min. 20 µl of the RT-PCR product was run in a 2% agarose gel in Tris-Borate-EDTA (TBE) buffer at 80 volts for 75 mins. The samples were visualised by ethidium bromide staining under UV light (Interscience) and photographed with Polaroid film (Fisher Scientific) (Fig. 10).

Table 4: Primer Sequences Used for RT-PCR

Primers	Sequence	Amplified Fragment
αIL-5Rm	5'-GAAATGATGAACACAAGCC -3' 5'-GTTAATTCTCTCGCTTATCTC 3'	92 bp
αIL-5Rs	5'-GGTTCTCAAGATAAAGG-3' 5'-CAACAGCAGGGATAGCAGATT-3'	84bp
β-Actin	5'-GGGTCAGAAGGATTCCTATG-3' 5'-GGTCTCAAACATGATCTGGG-3'	249 bp

4.5 Preparation of Probes

Non-specific cDNA encoding for both membrane-anchored and soluble isoforms of αIL-5R, cDNA specific for membrane-anchored αIL-5Rm isoform and cDNA specific for soluble αIL-5Rs were gifts from Dr. J. Tavernier (Roche Research, Brussels, Belgium). Non-specific cDNA encoding for both membrane-anchored and soluble isoforms of αIL-5R was in pCDM8 vector at *Xba* I site. The membrane-anchored specific

αIL-5R cDNA and soluble specific αIL-5R cDNA were in pCDM8 vectors at *BstX* I site. The 1074 bp non-specific cDNA for αIL-5R contained both membrane-bound and soluble segments (from 435 to 1509 bp), whereas the 92 bp αIL-5Rm riboprobe (from 1243 to 1335 bp) was specific for the membrane-anchored isoform (complementary to the exon containing the transmembrane region generated by alternative splicing) and the 84 bp αIL-5Rs riboprobe (from 1243 to 1327 bp) was specific for the soluble isoform (a soluble-specific exon containing essentially a non-coding region generated by normal splicing). These cDNA fragments were digested with the appropriate enzymes and then used as templates for construction of αIL-5R cRNA probes into a transcription vector, pGEM-7 (Promega). Figure 5 summarises the general procedures for subcloning the cDNA inserts of αIL-5R. Briefly, the insert and vector cDNA were prepared by restriction endonuclease digestion. The insert cDNA was ligated into pGEM vector, and then transformed into host strains of *E.coli*. Identification of subcloned transformants was done by isolation of plasmid DNA (mini-preparation). αIL-5R positive transformants were grown on a large scale. The αIL-5R cDNA probes in pGEM vectors were then linearized with appropriate restriction enzymes to produce antisense (having a complementary sequence to αIL-5R mRNA) or sense (having an identical sequence to αIL-5R mRNA) probes prior to the *in vitro* transcription with SP6 or T7 polymerase. Figures 6 and 7 illustrate the subcloning of αIL-5R cDNA inserts into a pGEM-7 transcription vector and restriction enzymes used for the preparation of riboprobes in detail.

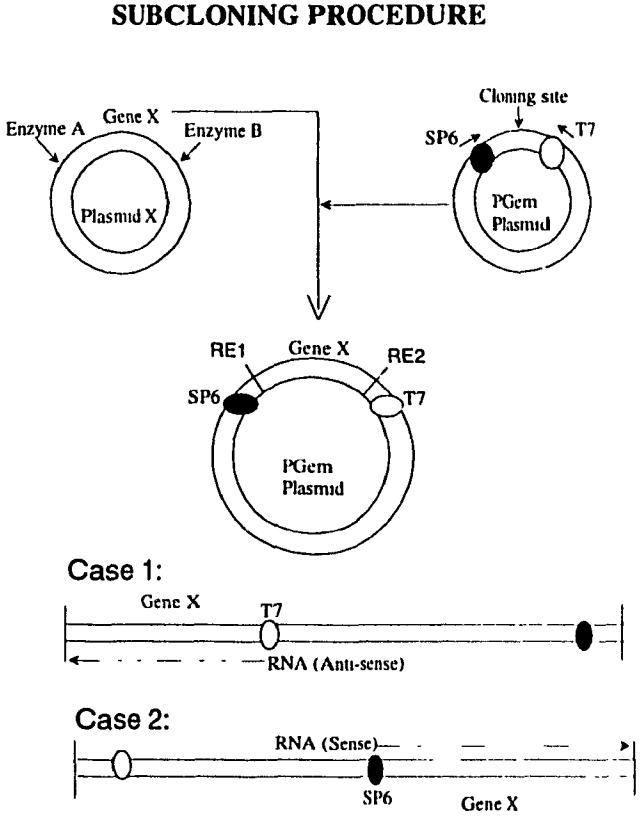

Fig. 5 : General procedure for subcloning the αIL-5 receptor cDNA insert. The cDNA to be transcribed is cloned into the cloning site of a transcription vector which contains a promoter site for SP6 and T7 RNA polymerase. Case 1 is achieved by digestion of the probe with restriction enzyme 1 (R.E.1) and then transcription with T7 RNA polymerase. Case 2 is achieved by digestion of the probe with restriction enzyme 2 (R.E.2) and then transcription with SP6 RNA polymerase.

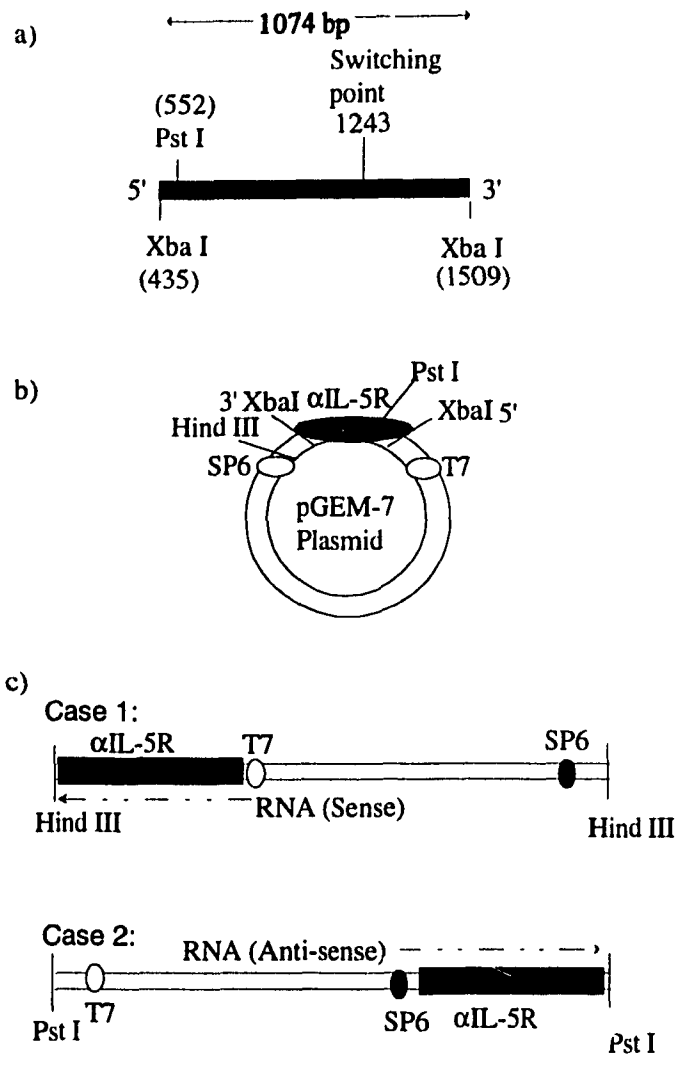

Fig. 6 :Preparation of the riboprobes from non-specific cDNA encoding for both membrane-anchored and soluble isoforms of αIL-5R. The restriction enzyme map of αIL-5R cDNA (1074 bp) is shown (a). αIL-5R cDNA was subcloned into pGEM-7 plasmid at *Xba* I site (b). Sense RNA probe was prepared by digestion of the probe with *Hind* III and then transcription with T7 RNA polymerase. Anti-sense RNA probe was prepared by digestion of the probe with *Pst* I and then transcription with SP6 RNA polymerase (c).

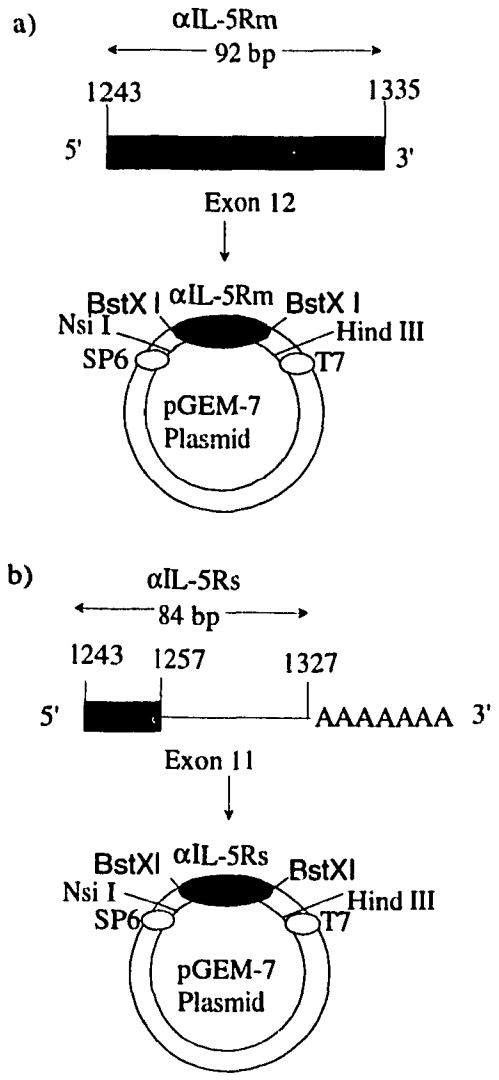

Fig. 7 : Preparation of the riboprobes from αIL-5Rm and αIL-5Rs cDNAs. αIL-5Rm cDNA specific for membrane-anchored isoform (92 bp long; coding region from 1243 to 1335 bp) was subcloned into pGEM-7 at *BstX* I site (a). αIL-5Rs cDNA specific for soluble isoform (84 bp long; essentially non-coding region from 1243 to 1327 bp) was subcloned into pGEM-7 at *BstX* I site (b). For both αIL-5Rm and αIL-5Rs, the sense probes were prepared by digestion of the probes with *Hind* III and then transcription with SP6 RNA polymerase, while anti-sense RNA probes were prepared by digestion of the probe with *Nsi* I and then transcription with T7 RNA polymerase.

4.6 Labelling riboprobes

Labelled antisense (cRNA) and sense transcripts of αIL-5R cDNA were synthesised by incubating 1.0 µg of linearized plasmid template in a 20 µl reaction mixture containing transcription buffer, 100 mM DTT (Promega), 1 Unit of RNase inhibitor (RNasin, Promega), 2.5 mM of ATP, GTP, and CTP (Promega), 29 pmol of ^{35}S-UTP (10 mCi/ml, Amersham), and 10 U of SP6 or T7 RNA polymerase (Promega). The above reaction mixture was then incubated at 37°C for 60 minutes. The cDNA template was removed by adding 1 µl of RNase-free DNase (1 µg/µl, Promega) for 10 minutes at 37°C. The RNA probe was purified by extraction with equal volume of phenol/ chloroform (Gibco-BRL). The probes were precipitated in 100% ethanol containing 2.5 M ammonium acetate at -20°C overnight and were centrifuged (12,000 rpm for 30 minutes) the following day. The pellet was then washed with 70% prechilled ethanol, vacuum dried for 15 minutes, and dissolved in 20 µl of DEPC-H_2O. 1µl of the probes were taken for β-counting, while the rest were kept at -80°C and used as soon as possible.

4.7 In situ hybridisation

For *in situ hybridisation*, cryostat sections (6 µm) were cut from the biopsies, mounted on 0.1% poly-L-lysine coated slides (Sigma) (to ensure the tissues remained fixed to the slide), and allowed to dry for 12 h at 37 °C before processing for hybridisation. To increase the efficiency of hybridisation by rendering the target sequences more accessible to the probe, the preparations were permeablized prior to the application of the probe with a 0.3 % solution of

Triton X-100 in PBS for 10 min at room temperature and then with a proteinase K solution (1 µg/ml in 20 mM Tris-HCl and 1 mM EDTA, pH 7.2) for 15 min at 37 °C. The reaction was terminated by immersion of the preparations in 4% paraformaldehyde for 5 min. The slides were then treated in 0.25 % acetic anhydride in 0.1 M triethanolamine (Sigma) for 10 min at 40°C to reduce non-specific binding of the ^{35}S-labelled complementary RNA probes. The prehybridisation of the preparations was carried out in 50 % formamide and 2 x standard saline citrate (SSC) (for 10 × SSC: 0.15 M of sodium chloride, 0.15 M of sodium citrate, pH 7.0) for 30 min at 40 °C. Hybridisation was performed using a hybridisation mixture containing 4.5 ng (0.75×10^6 cpm) of the appropriate IL-5Rα sense and antisense probes per section in hybridisation buffer (50% deionized formamide, 5 × SSC, 10% dextran sulphate (Sigma), 5 × Denhardt's solution (Sigma), 0.5% SDS (Sigma), 100 µg/ml denatured salmon sperm DNA (Sigma) and 100 mM DTT (Sigma)). The preparations were covered with dimethyldichlorosilane-coated coverslips and hybridisation was carried out in a humid chamber for 16 h at 40 °C. Posthybridisation washing was performed under high stringency conditions by using decreasing concentrations of SSC (4 x SSC to 0.05 x SSC) at 45 °C for 20 min. Unbound, single-stranded RNA was removed by treating the preparations with a solution containing RNase A (20 µg/ml RNase A, 0.5 M NaCl, 10 mM Tris-HCl pH 8.0, and 1 mM EDTA) for 30 min at 37 °C. The samples were then dehydrated in 70%, 95% and 100% ethanol containing 0.3 M ammonium acetate for 5 minutes each, then air-dried. The sections were then immersed in K-5 liquid emulsion at 43°C (Amersham) in the dark room and then air-dried. The slides were exposed in a light-tight box for 14 days at 4°C. The

autoradiographs were then developed in a developer (D-19, Kodak) for 3 minutes in the dark room at 17°C, washed in dH_2O for 3 seconds, and fixed in the fixer hypam (Ilford) for 5 minutes at 17°C. The slides were washed in tap water for at least 10 minutes and counterstained with haematoxylin (BDH) for 2 minutes. The slides were dehydrated in 70%, 90% and 100% alcohol for 5 minutes each, immersed in Xylene (BDH) for 5 minutes, mounted with Entellan mountant (VWR), overlaid with cover slips, and then examined under a graduated microscope for positive signals. Control experiments were carried out by hybridisation of the corresponding sense probe and, in another set of preparations, by treatment of the slides with RNase A solution (50 μg/ml) before hybridisation with antisense probe.

Specific hybridisation was visualised as deposits of silver grains in the photographic emulsion overlaying the tissue samples. Cells were determined by visualising the individual nuclei using dark field illumination. Positive cells were only observed by hybridisation with an antisense probe; samples treated with sense probes or pre-treated with RNase were negative.

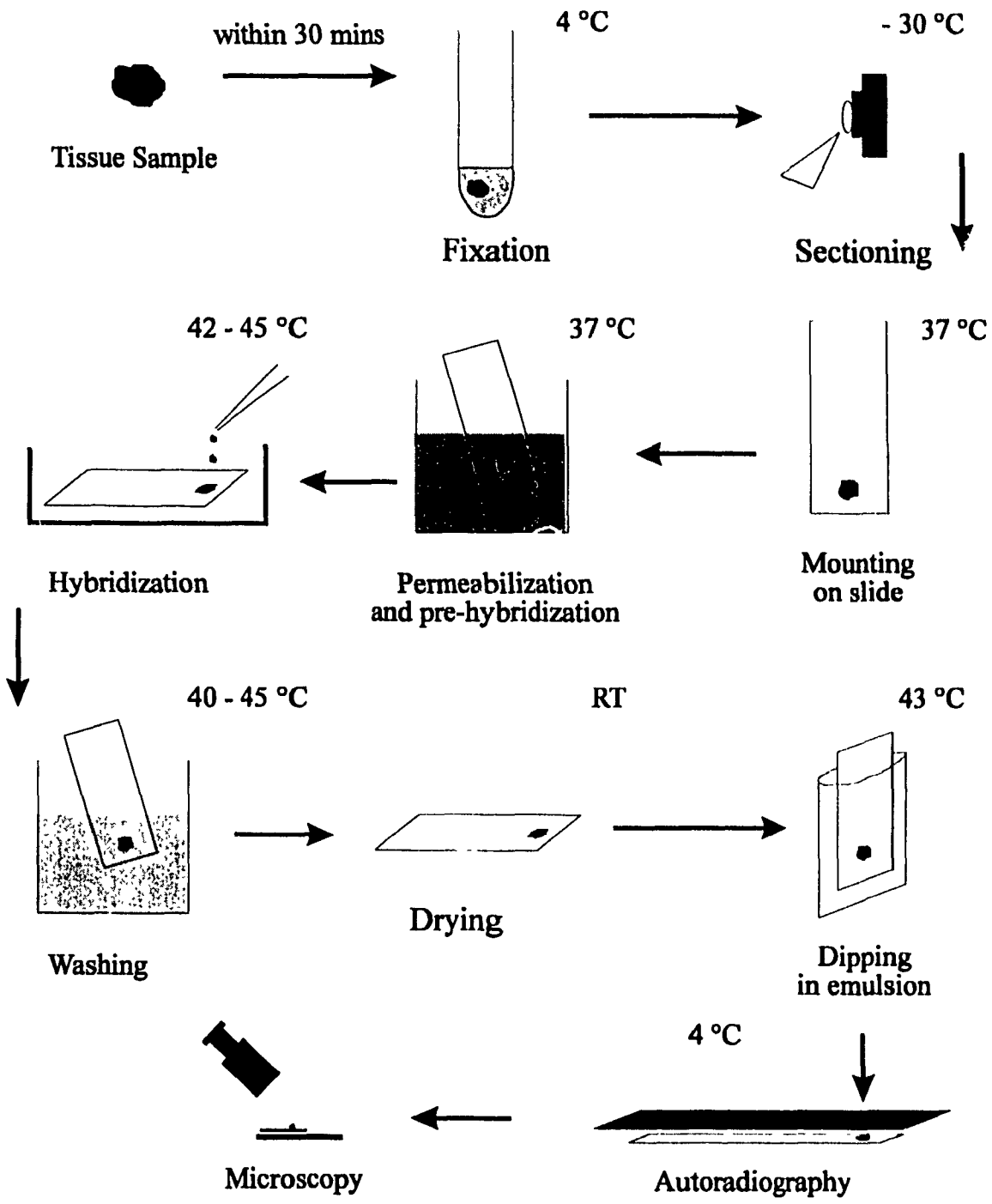

Fig. 8 : Schematic representation of general *in situ* hybridisation technique.

4.8 Simultaneous in situ hybridisation and immunocytochemistry

Modified alkaline phosphatase anti-alkaline phosphatase (APAAP) technique was used to phenotype cells (Frew & Kay, 1988) (Fig. 12). Briefly, slides were immersed in TBS (150 mM NaCl, 50 mM Tris-HCl, pH 7.4) for 5 min, then incubated for 30 min with mouse primary monoclonal antibody EG2 (1:100 dilution) (Pharmacia), which recognises human activated eosinophils. Slides were then incubated with a rabbit anti-mouse Ig (Dako) and APAAP conjugates (Dako) for 30 min each. Rabbit anti-mouse Ig and APAAP conjugates were diluted 1/30 with 20% human normal serum. Washings were performed between all steps (3 × 2 min) with TBS. All incubations were carried out at room temperature. In order to avoid RNase contamination, all solutions used were prepared in 0.1% DEPC-treated H_2O. Additionally, the 20% human serum used also contained 100 U/ml of RNase inhibitor (RNasin). To develop the reaction, Fast Red TR (1 mg/ml in 0.1 M Tris buffer containing 0.2 mg/ml of Naphthol-As-Mx-Phosphate (Sigma) and 0.24 mg/ml of Levamisole, pH 8.2 (Sigma)) was used as the chromogen and the intensity of the immunostaining reaction was adjusted by light microscopy. The reaction was stopped by washing in TBS, then in PBS. For negative control preparations, the primary antibody was replaced with TBS. After immunochemical staining, the same slides were processed for *in situ hybridisation* using radiolabeled αIL5R cRNA probe as described earlier. Sections were counterstained with haematoxylin (BDH) and mounted in Crystal mounting medium (ESBE Scientific).

ICC positive cells were stained red while ISH positive signals were localised as a dense collection of silver grains overlying cells (black grains under bright field and white grains under dark field).

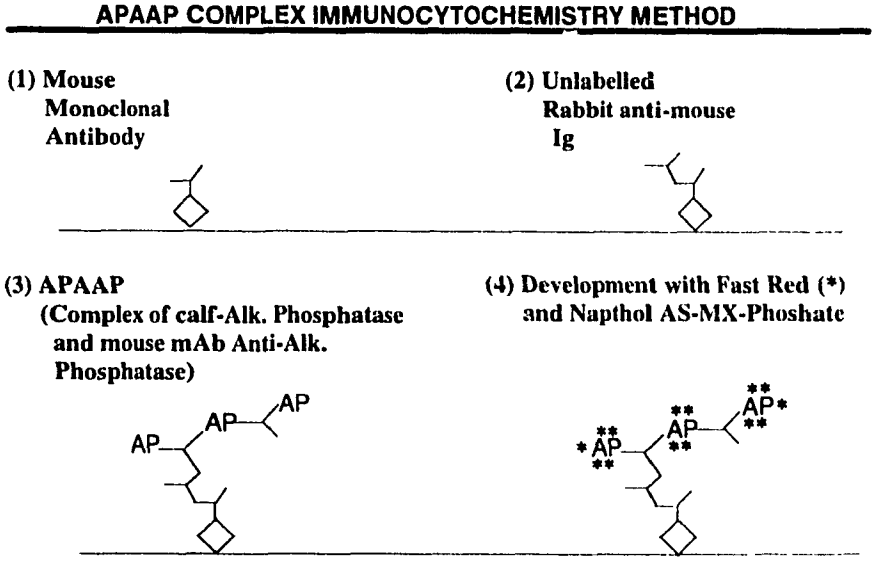

Fig. 9 : Diagram of Immunocytochemistry using the Alkaline Phosphatase Anti-Alkaline Phosphatase (APAAP) technique.

4.9 Quantification

Positive cells for αIL-5R were counted in a 115-mm zone along the entire length of epithelial basement membrane using a light microscope with 100 x magnification with an eye-piece graticule (0.202 mm^2). At least two sections and a median of four fields were counted for cells positive for ISH and simultaneous ICC and ISH. Positively staining cell counts were expressed as the mean number of positive cells per mm basement membrane. The number of cells co-expressing αIL-5R mRNA and EG2 was presented as a percentage of the total cells expressing αIL-5R mRNA.

4.10 Statistical Analysis

Comparisons between cell counts of asthmatic and control biopsies were performed using the Mann-Whitney test for unpaired samples with Bonferroni corrections for multiple testing. They were also tested for significance using the Kruskal-Wallis analysis of variance for non-parametric data. Correlations were obtained using the Pearson correlation co-efficient. Results were considered statistically significant for $p < 0.05$ (Glantz, 1992).

5.0 RESULTS

The clinical features of the 19 stable asthmatics (atopic=9; intrinsic=10) and 20 non-asthmatic controls (atopic=10 ; non-atopic=10) who were studied are presented in Tables 5 and 7.

RT-PCR technique using specific primers confirmed the expression of membrane-anchored and soluble isoform of αIL-5R mRNA (αIL-5Rm and αIL-5Rs) in biopsies from asthmatic patients (92 bp band, lane 2; 84 bp band, lane 5) but not from control subjects (lane 3; lane 6) (Fig. 10). β-actin was used as a "housekeeping" gene, which is constitutively expressed in order to overcome variability in RNA purification. Similar expression of β-actin was found in both asthmatic as well as control subjects (249 bp band lane 8; 249 bp band lane 9) (Fig. 10). αIL-5R, αIL-5Rm and αIL-5Rs mRNA were localised at the tissue level by *in situ* hybridisation using cRNA probes. Preparation of the riboprobes from non-specific αIL-5R cDNA which recognized both membrane anchored and soluble isoforms (1074 bp) was done by subcloning the cDNA into a pGEM-7 transcription vector at the *Xba* I site (Fig. 6). The restriction enzyme map of the αIL-5R cDNA insert is shown (Fig. 6a). Sense and antisense probes were prepared by digestion of the probe appropriate restriction enzymes and then transcription with either T7 or SP6 RNA polymerases (Fig. 6c). Preparation of the riboprobes from αIL-5Rm cDNA specific for membrane-anchored isoform (92 bp long; from 1243 to 1335 bp) was done by subcloning the cDNA into pGEM-7 at the *BstX* I site (Fig. 7a). The preparation of the riboprobes for the αIL-5Rs

was performed by subcloning the soluble specific cDNA (84 bp long; from 1243 to 1327 bp) into pGEM-7 at the *BstX* I site (Fig. 7b). For both αIL-5Rm and αIL-5Rs, the sense and antisense probes were prepared by digestion of the probes with appropriate restriction enzymes then transcription with either SP6 or T7 RNA polymerase.

Hybridisation between the labelled cRNA probes and αIL-5R mRNA was detected by specific intense deposits of silver grains in the photographic emulsion overlying the tissue samples from asthmatics (Fig. 11a) but not in control subjects (Fig. 11c). The specificities of the probe and of the hybridisation were confirmed either by the absence of hybridisation signals when all the preparations were treated with a sense probe (Fig. 11b) or after pre-treatment of the preparations with RNase A prior to labelled αIL-5R cRNA application (Fig 11d). Cells with positive hybridisation signals were localised mainly below the basement membrane. The positive cells were round and relatively large, compatible with the shape and size of eosinophils (Fig. 11a). The number of αIL-5R mRNA positive cells varied from 4.8 to 18.0 /mm of basement membrane in asthmatics (Table 5). There were no hybridisation signals on epithelial nor endothelial cells. The asthmatic biopsies had more positive cells/mm basement membrane than control subjects. The number of positive αIL-5R mRNA cells in atopic (8.77±1.54) and non-atopic asthmatic biopsies (11.12±1.40) and in atopic (0.85 ±0.33) and non-atopic controls (1.00 ±0.40) are shown in Table 7. There was a trend that the patients who were αIL-5R mRNA-positive had lower FEV_1 percent predicted (range 56-112) and PC_{20} methacholine (0.02-3.66) compared to controls who were αIL-5R mRNA negative (FEV_1 81-125; PC_{20} 7.61->16) (Table 5).

The number of positive cells/mm basement membrane expressing mRNA for αIL-5R were significantly higher in asthmatic biopsies than in non-asthmatic controls ($p<0.001$) (Fig. 13). There was a significant increase in the numbers of cells/mm basement membrane expressing mRNA for both αIL-5Rm and αIL-5Rs isoforms in the bronchial biopsies of asthmatics compared to controls ($p<0.001$) (Fig. 15 & Fig. 17). There was no significant difference in the expression of αIL-5Rm and αIL-5Rs between atopic and non-atopic asthmatics who had been matched for their severity of asthma. In addition, there was no significant difference between the expression αIL-5Rm and αIL-5Rs between atopic and non-atopic controls. Interestingly, in asthmatic biopsies, the number of positive cells expressing mRNA for total αIL-5R correlated inversely with FEV_1 ($r^2 =0.77$, $p<0.001$)(Fig. 14). However, in the same subjects, the number of positive cells expressing mRNA for αIL-5Rm correlated inversely with FEV_1 ($r^2 =0.89$, $p<0.001$), whereas the expression of αIL-5Rs mRNA correlated directly with FEV_1 ($r^2 =0.52$, $p<0.001$) (Fig. 16 & 18). Simultaneous hybridisation of the αIL-5R probe and immunostaining with EG2 antibody on bronchial biopsies from asthmatics revealed that 93% (±2.3) of the cells which were αIL-5R mRNA positive were also EG2 positive (Fig. 12) (Table 8). This indicated that the major site of αIL-5R synthesis was activated eosinophils. In addition, there was a significant inverse correlation between αIL-5Rm and αIL-5Rs mRNA ($r^2=0.43$, $p < 0.005$) (Fig. 19). No significant correlations were observed between αIL-5R isoforms and airway responsiveness as measured by methacholine PC_{20}

Table 5: Patient Characteristics

Subject Number	Subject	Age/Sex (year)	IgE (U/L)	FEV_1 (%)	PC_{20} (mg/ml)	Disease Duration (Years)
1	NANC	32/F	16	110	>16	N/A
2	NANC	39/F	7	110	>16	N/A
3	NANC	25/F	<4	119	>16	N/A
4	NANC	23/M	18	90	>16	N/A
5	NANC	21/M	29	92	>16	N/A
6	NANC	42/M	5	100	>16	N/A
7	NANC	21/M	5	118	>16	N/A
8	NANC	21/F	<4	106	>16	N/A
9	NANC	20/M	6	99	>16	N/A
10	NANC	19/F	35	109	>16	N/A
11	ANC	22/M	107	125	>16	N/A
12	ANC	22/M	107	125	>16	N/A
13	ANC	27/M	151	81	>16	N/A
14	ANC	33/M	152	123	>16	N/A
15	ANC	25/F	808	91	7.61	N/A
16	ANC	24/M	109	92	>16	N/A
17	ANC	21/M	241	121	>6	N/A
18	ANC	28/M	83	100	>16	N/A
19	ANC	36/M	38	100	>16	N/A
20	ANC	23/M	77	107	>16	N/A
21	AA	24/M	253	72	0.07	4
22	AA	26/M	251	79	0.02	25
23	AA	28/F	57	100	2.99	8
24	AA	42/M	330	85	0.14	7
25	AA	39/M	374	81	3.45	>30
26	AA	36/F	62	100	3.61	>30
27	AA	55/M	180	56	0.16	>30
28	AA	26/M	78	86	3.66	20
29	AA	42/M	295	104	1.63	20
30	IA	49/F	29	96	1.34	12
31	IA	54/F	46	78	6.00	11
32	IA	52/F	108	64	2.04	16
33	IA	54/F	108	73	ND	12
34	IA	46/M	99	70	ND	12
35	IA	61/M	17	58	0.25	16
36	IA	62/M	75	61	0.50	8
37	IA	58/M	40	12	1.00	3
38	IA	39/F	33	102	0.12	1
39	IA	53/F	79	92	0.12	10

NANC: Non -Atopic Normal controls; ANC: Atopic Normal Control; AA: Atopic Asthmatics; IA: Intrinsic Asthmatics

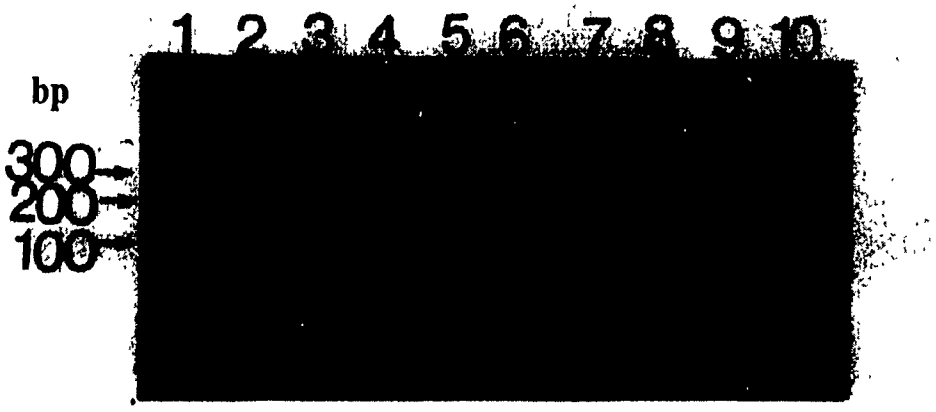

Fig. 10 : Analysis of αIL5Rm and αIL5Rs mRNA by RT-PCR procedure. After the reverse transcription step, the cDNA was amplified using specific primers. The RT-PCR products were run in a 2% agarose gel and then stained with ethidium bromide. Lane 1: 100 bp DNA ladder; 2: αIL5Rm PCR product in atopic asthmatics; 3: αIL5Rm PCR product in non-asthmatic control; 4: αIL5Rm primers in water (negative control); 5: αIL5Rs PCR product in atopic asthmatic; 6:αIL5Rs PCR product in non-asthmatic control; 7: αIL5Rs primers in water; 8: β-Actin gene expression in asthmatics; 9: β-Actin gene expression in non-asthmatic control; 10: β-Actin primers in water.

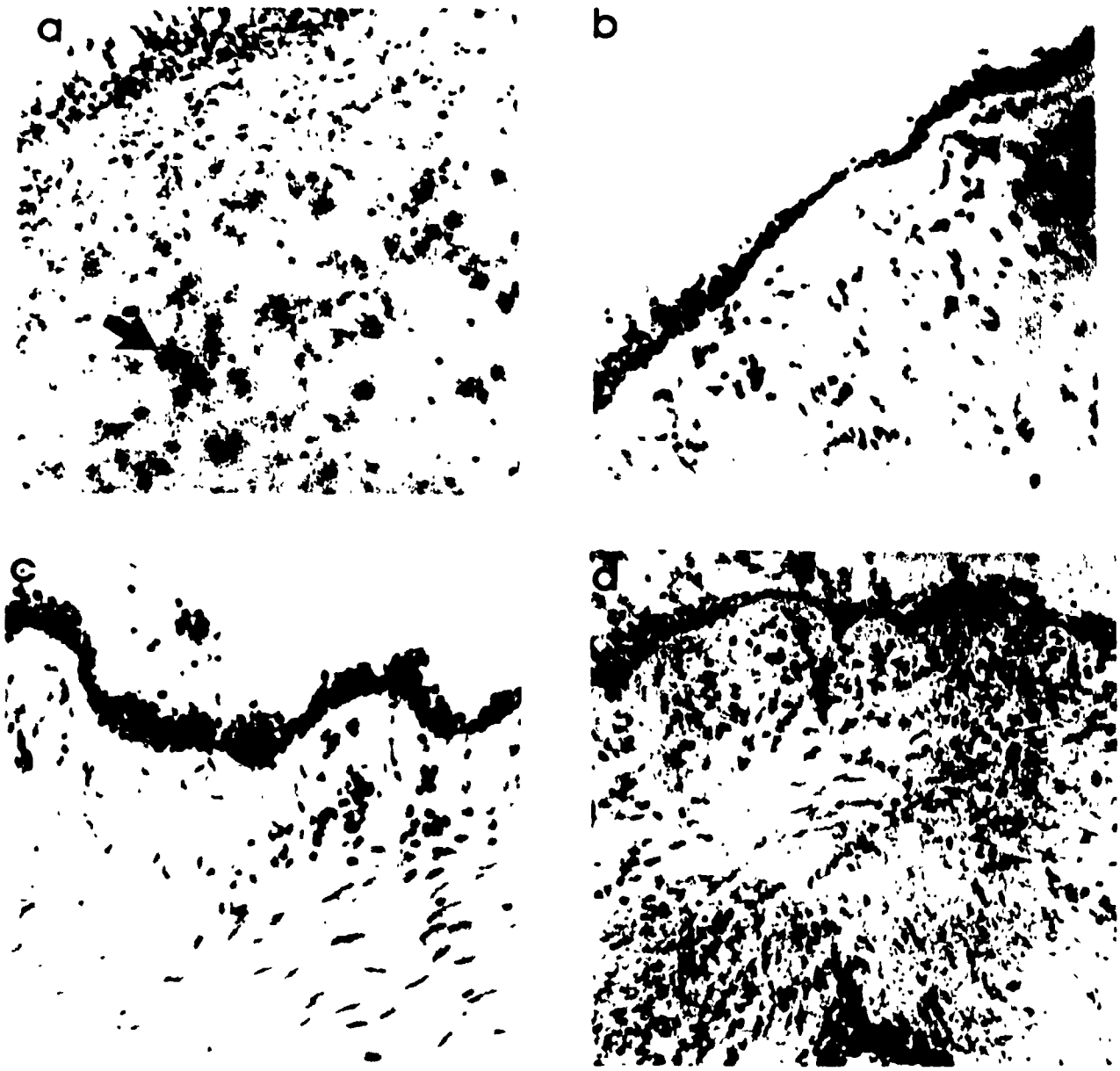

Fig. 11 : Typical *in situ* hybridisation autoradiographs. Positive hybridisation signals are observed using ^{35}S-labelled αIL-5Rm cRNA probe in a cryostat section of bronchial mucosa from a patient with asthma (a). Arrow indicate the positive αIL-5R mRNA cells. No signal was seen in bronchial mucosa from asthmatics with the αIL-5R sense probe (b), nor in pre-treatment with RNase (d). No hybridisation signal was detected in bronchial mucosa from normal subject (c). Magnification X 250.

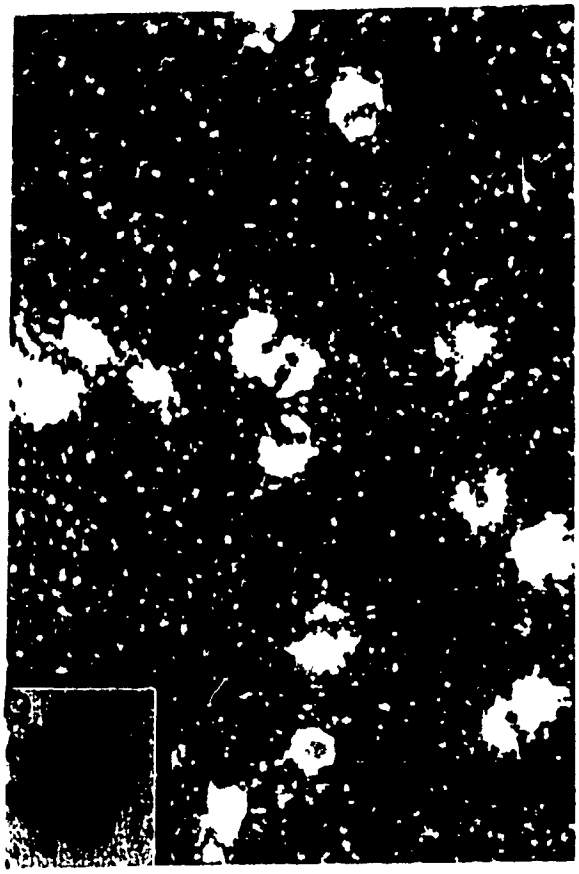

Fig. 12 : Typical simultaneous *in situ* hybridisation and immunocytochemistry autoradiograph. (a) dark field illumination of ^{35}S-labelled αIL-5R cRNA probe positive hybridisation signals in a cryostat section of bronchial mucosa from a patient with asthma in which the arrow indicates the positive αIL-5R mRNA cells. Magnification X 400. *Insert* (b), simultaneous *in situ* hybridisation and immunocytochemistry autoradiographs using ^{35}S-labelled αIL-5R cRNA probe and APAAP. Immunostaining with EG2 confirmed that most of the αIL-5R mRNA positive cells were eosinophils in asthmatic biopsies. Magnification X 1000.

Table 6: Comparison of *In situ* hybridisation data on IL-5 Receptor isoforms and clinical features

Subject no.	Subject	PC_{20}	FEV_1	αIL5R	αIL5Rm	αIL5Rs
1	NANC	>16	110	0	1.3	0.5
2	NANC	>16	110	1.5	0	0
3	NANC	>16	119	0	0	1
4	NANC	>16	90	4	0	2
5	NANC	>16	92	1	2.3	1.5
6	NANC	>16	100	0	1.5	0.5
7	NANC	>16	118	0.5	0	0.5
8	NANC	>16	106	2	1	1
9	NANC	>16	99	1	0	0
10	NANC	>16	109	0	0.5	2
11	ANC	>16	125	0	1	2
12	ANC	>16	125	1.5	1	2
13	ANC	>16	81	0	0.5	0.5
14	ANC	>16	123	0.5	1	0.3
15	ANC	7.61	91	2	1.5	0.3
16	ANC	>16	92	3	2.3	1.5
17	ANC	>16	121	0	0	0
18	ANC	>16	100	0.5	0.3	1
19	ANC	>16	100	0	0	0
20	ANC	>16	107	1	2	1.3
21	AA	0.07	72	9.5	12.3	4.6
22	AA	0.02	79	12.9	10.9	5.9
23	AA	2.99	100	5	5.5	8.5
24	AA	0.14	85	6.5	4.9	6.2
25	AA	3.45	81	7.5	9.2	8.3
26	AA	3.61	100	4.5	3.1	8.1
27	AA	0.16	56	17.5	16.5	2.6
28	AA	3.66	86	12	6.5	8.7
29	AA	1.63	104	3.5	2	6.3
30	IA	1.34	96	7.2	5.5	7.4
31	IA	6	78	11.7	10.1	5.7
32	IA	2.04	64	18	15	7.3
33	IA	ND	73	11.9	12.3	8.1
34	IA	ND	70	15.3	12	7
35	IA	0.25	58	16.5	18.2	4.1
36	IA	0.5	61	10.9	13	4
37	IA	1	112	4.8	4	10
38	IA	0.12	102	7.1	3	8
39	IA	0.12	92	7.8	7.1	8

NANC: Non -Atopic Normal controls; ANC: Atopic Normal Control; AA: Atopic Asthmatics; IA: Intrinsic Asthmatics

PC_{20} : mg/ml

FEV_1 : %

αIL5R, αIL5Rm and αIL5Rs: number of mRNA+ve cells/mm BM

Table 7: Summary of Clinical features and Experimental Data

	Non-Atopic Normal control	Atopic Normal control	Atopic Asthma	Intrinsic Asthma
Age (yr)	52.80(±2.20)	35.33(±3.43)	26.30(±2.65)	26.10(±1.58)
Sex (F:M)	6:4	1:9	2:7	6:4
FEV_1 (%)	105.3(± 3.14)	106.50(± 5.11)	84.78(± 5.10)	80.60 (± 5.92)
PC_{20} (mg/ml)	N/A	N/A	1.75(± 0.56)	1.42(± 0.70)
No of αIL-5R mRNA +ve cells/ mm BM	1.00(±0.40)	0.85(±0.33)	8.77(±1.54)	11.12(±1.40)
No of αIL-5Rm mRNA+ve cells/mm BM	0.66(±0.26)	0.96(±0.25)	7.88(±1.58)	10.02(±1.58)
No of αIL-5Rs mRNA+ve cells/mm BM	0.90(±0.23)	0.89(±0.25)	6.58(±0.69)	6.96(±0.59)

Values are expressed as Mean ± S.E.

Table 8: Simultaneous *in situ* hybridisation and immunocytochemistry experimental data

Subjects	Total number of cells positive for αIL-5R mRNA /section	Number of EO +ve for αIL-5R mRNA /section	% of EO +ve for αIL-5R mRNA /section	Average number of total cells +ve for αIL-5R mRNA /section	Average number of EO +ve for αIL-5R mRNA /section	Average % of EO +ve for αIL-5R mRNA /section
AA	45	40	89			
AA	42	36	86			
AA	36	34	96	43.0 (±4.8)*	39.6 (±4.6)*	92.8 (±2.3)*
IA	60	57	95			
IA	32	31	98			

AA: Atopic Asthmatics; IA:Intrinsic Asthmatics; EO: Eosinophil .
*Numbers are expressed as Mean ±S.E.

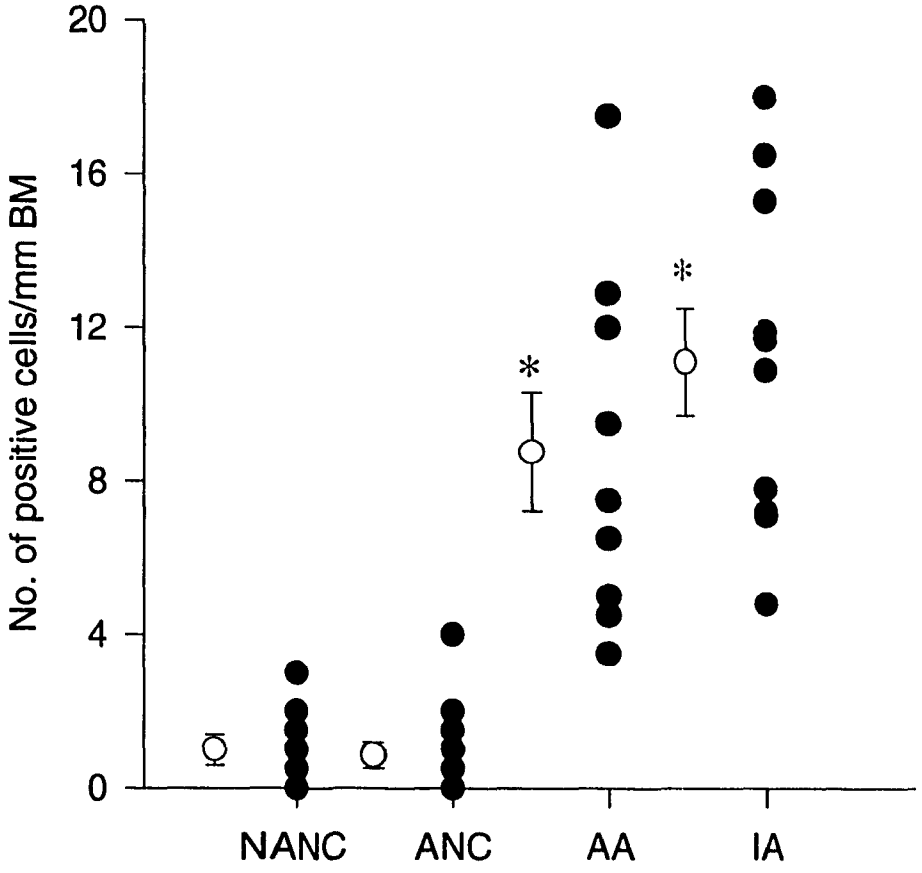

Fig. 13 : Number of αIL-5R mRNA positive cells in bronchial biopsies from asthmatics (atopic asthmatics n=9 and intrinsic asthmatics n=10) and controls (atopic normal control n=10 and non-atopic normal control n=10). Open circles represent the mean and the vertical lines indicate upper and lower limits of the s.e. mean. αIL-5R mRNA positive cells/mm basement membrane were significantly increased in asthmatic subjects compared to the controls (* $p<0.05$; Mann-Whitney test with Benferroni corrections).

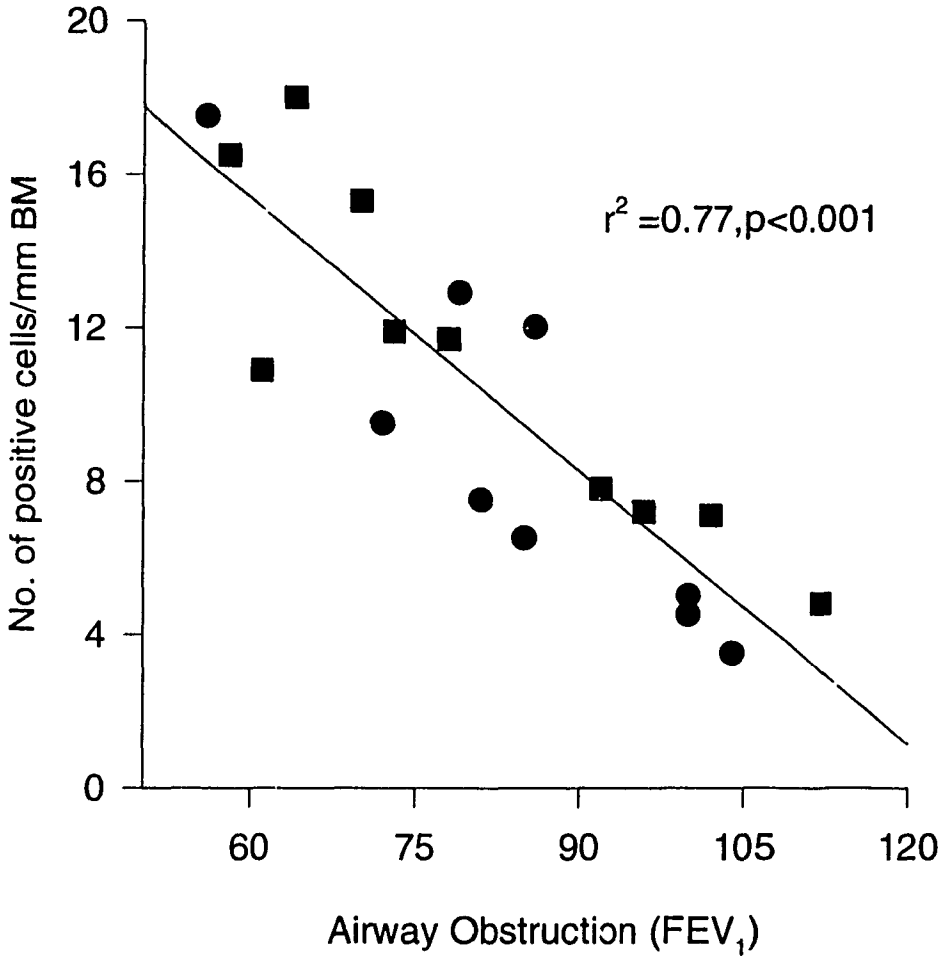

Fig. 14 : Correlation of number of αIL-5R mRNA positive cells with FEV_1 in atopic asthmatics (circles; n=9) and intrinsic asthmatics (squares; n=10). The number of αIL-5R mRNA positive cells were inversely correlated with FEV_1 (r^2=0.77, p<0.001, Pearson correlation coefficient).

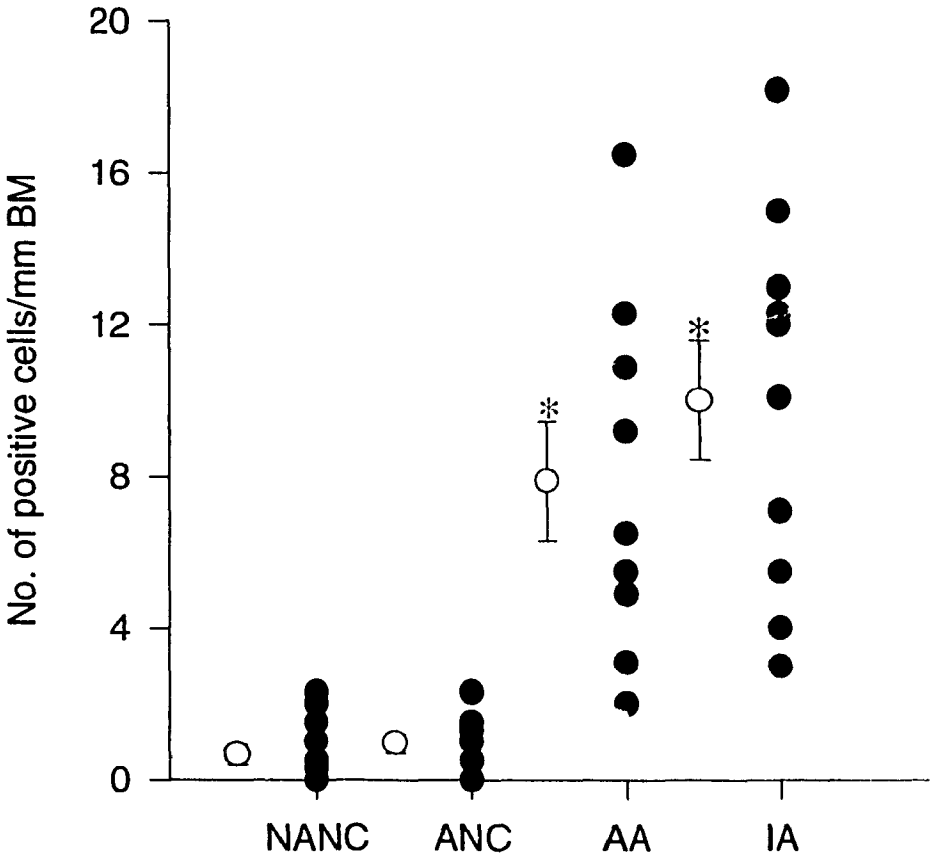

Fig. 15 : Number of αIL-5Rm mRNA positive cells in bronchial biopsies from asthmatics (atopic asthmatics n=9 and intrinsic asthmatics n=10) and controls (atopic normal control n=10 and non atopic normal control n=10). Open circles represent the mean and the vertical lines indicate upper and lower limits of the s.e. mean. αIL-5Rm mRNA positive cells/mm basement membrane were significantly increased in asthmatic subjects compared to the controls (* $p<0.05$; Mann-Whitney test with Benferroni corrections).

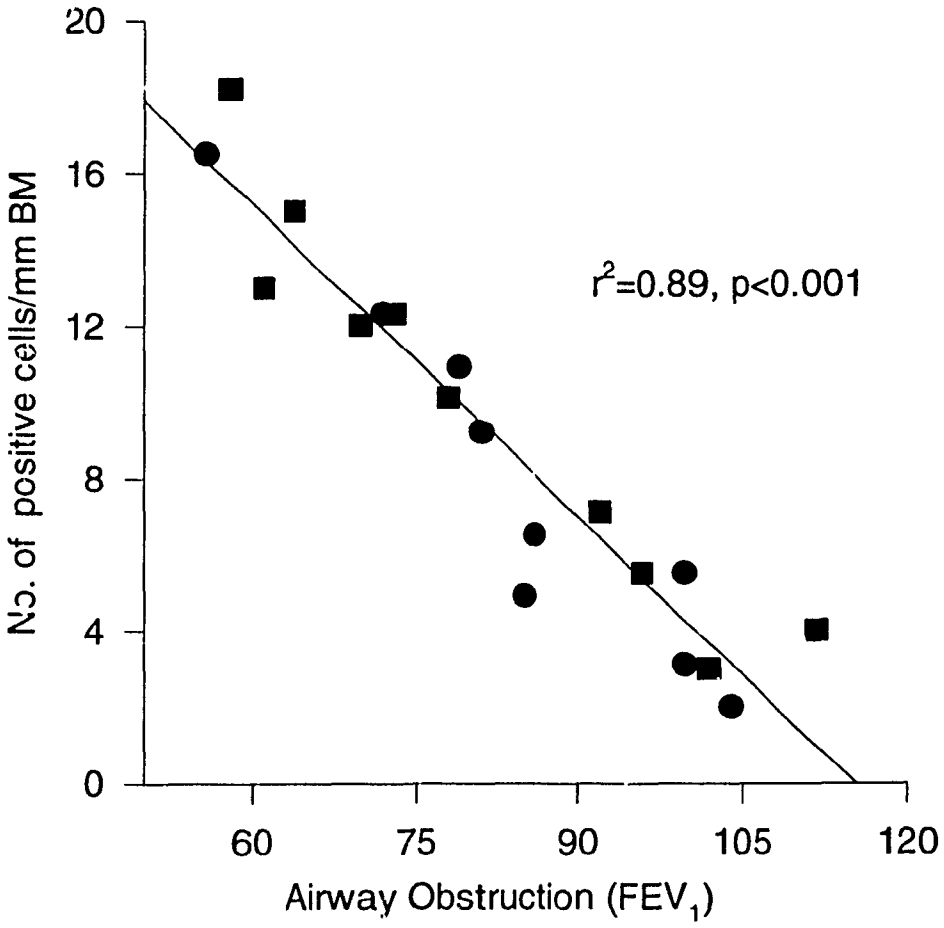

Fig. 16 : Correlation between the number of αIL-5Rm mRNA positive cells with FEV_1 in atopic asthmatics (circles; n=9) and intrinsic asthmatics (squares; n=10). The number of αIL-5Rm mRNA positive cells was inversely correlated with FEV_1 (r^2=0.89, p<0.001, Pearson correlation coefficient).

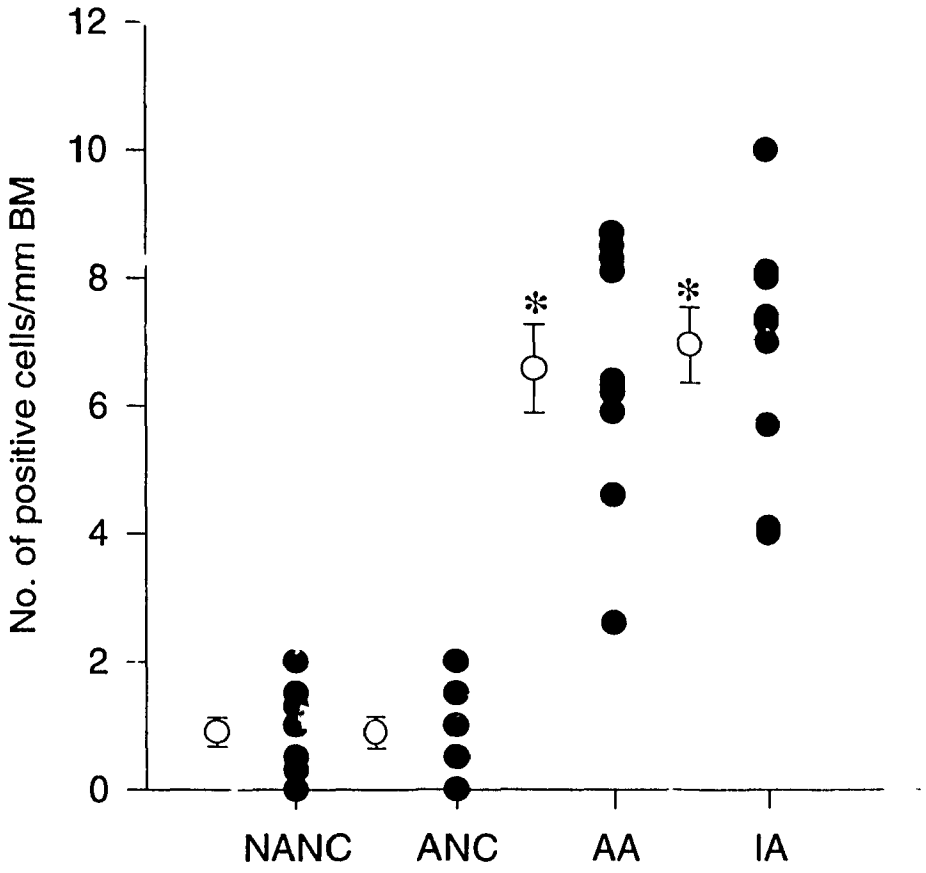

Fig. 17 : Number of αIL-5Rs mRNA positive cells in bronchial biopsies from asthmatics (atopic asthmatics n=9 and intrinsic asthmatics n=10) and controls (atopic normal control n=10 and non-atopic normal control n=10). Open circles represent the mean and the vertical lines indicate upper and lower limits of the s.e. mean. αIL-5Rs mRNA positive cells /mm basement membrane were significantly increased in asthmatic biopsies compared to the controls (* $p<0.05$; Mann-Whitney test with Benferroni corrections).

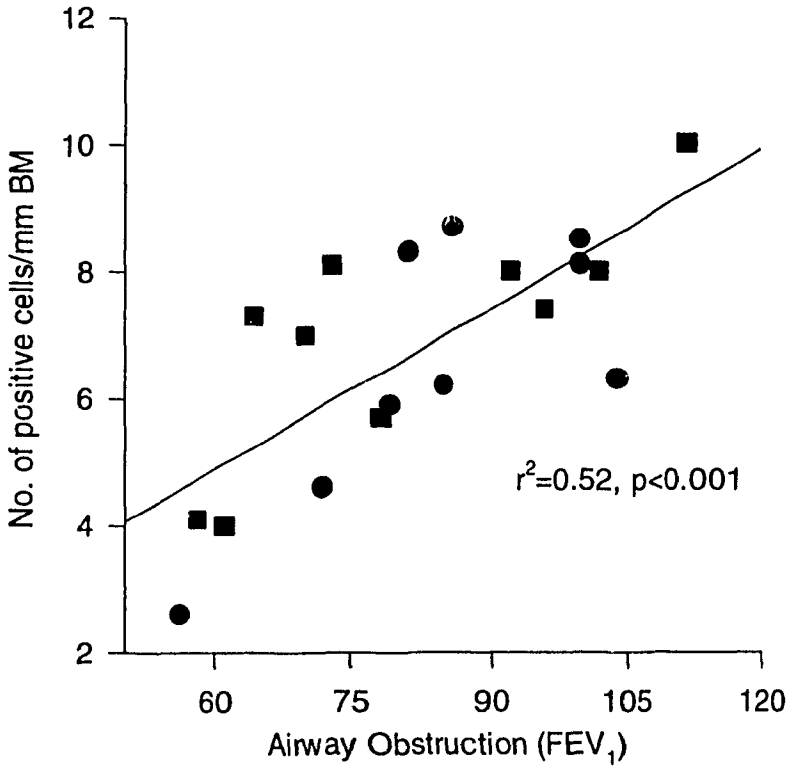

Fig. 18 : Correlation of the number of αIL-5Rs mRNA positive cells with the clinical feature of FEV_1 in atopic asthmatics (circles; n=9) and intrinsic asthmatics (squares; n=10). The number of αIL-5Rm mRNA positive cells were directly correlated with FEV_1 (r^2=0.52, p<0.001, Pearson correlation coefficient).

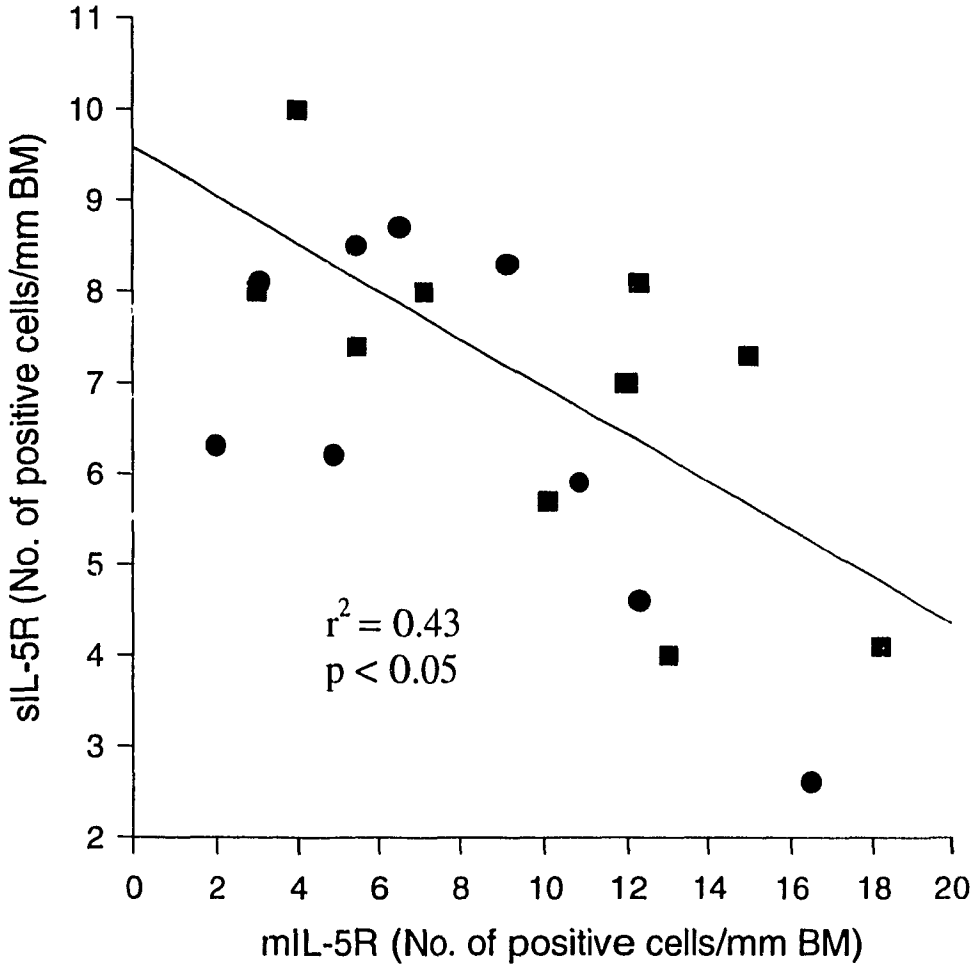

Fig. 19 : Correlation between soluble and membrane bound αIL-5R mRNA expression in atopic asthmatics (circles; n=9) and intrinsic asthmatics (squares; n=10). The number of soluble αIL-5R mRNA positive cells was inversely correlated with the number of membrane-anchored αIL-5R mRNA positive cells (r^2= 0.43, $p<0.05$, Pearson correlation coefficient).

6.0 DISCUSSION

This study presents evidence for an increase in the number of αIL-5R mRNA positive cells in bronchial biopsies of atopic and non-atopic asthmatic individuals compared to atopic and non-atopic controls. The enhanced expression of αIL5R mRNA in asthmatics was predominantly associated with eosinophil number. These observations parallel previous reports which detected IL-5 mRNA expression in bronchial biopsy samples from atopic and non-atopic (intrinsic) asthmatic patients and which found that IL-5 expression inversely correlates with pulmonary function (Hamid et al., 1991; Robinson et al., 1993; Ying et al., 1995). It has also been shown that the number of IL-5 mRNA-expressing cells directly correlated with the degree of tissue eosinophilia in asthmatic airways (Bentley et al 1993). Activated T lymphocytes have been shown to secrete IL-5 (Walker et al., 1992; Ying et al., 1995) and IL-5 has been shown to recruit and activate eosinophils *in vitro* (Walsh et al., 1990; Lopez et al., 1988). It was suggested that the IL-5 activated eosinophils would in turn secrete cytotoxic granule mediators to cause significant histopathological alterations in the mucosa of asthmatics by damaging the airway epithelium, by promoting further development of airway inflammation, and by causing airway hyperresponsiveness.

These previous reports led to the hypotheses of this thesis that there is an increase in local production of αIL-5R mRNA in the bronchial walls of asthmatics, that eosinophils are the major site of αIL-5R synthesis, and that there is differential expression of membrane bound and soluble αIL-5R isoforms. This thesis presents evidence to support previous findings

that IL-5 may act on eosinophils by interacting with a specific membrane IL-5 receptor which is expressed primarily by eosinophils from the asthmatic mucosa.

The localisation of αIL-5R mRNA was demonstrated by the technique of *in situ* hybridisation. The αIL-5R cDNAs had been previously characterised and utilised for detection of αIL-5R mRNA (Tavernier et al., 1992). The specificity of the hybridisation was further validated for this study by using αIL-5R sense probes and pre-treatment of the samples with RNase. The presence of strong hybridisation signals, the stability of the signal after high-stringency washing conditions, and the absence of hybridisation with either sense probes or RNase pre-treatment confirmed the specificity of both the probes as well as of the hybridisation signals for αIL-5R mRNA (Hamid et al., 1991).

The cDNA encoding human αIL-5R has been isolated from several cell lines, including eosinophils *in vitro* (Lopez et al., 1991). However, αIL-5R mRNA has never been previously demonstrated in eosinophils from the bronchial mucosa of asthmatics. In this investigation, co-localisation studies demonstrated that 93%(±2.3) of αIL-5R mRNA positive cells from asthmatic biopsies were activated eosinophils (EG2 positive). The cDNA used for generating the cRNA probe in this co-localization study detected both soluble and membrane-bound isoforms of αIL-5R, but did not differentiate between the two. This finding provides evidence that IL-5 may specifically stimulate eosinophils through the specific membrane-anchored α-subunit of its receptor on the surface of these cells. These results agree with previous reports which showed that the lineage specificity of IL-5 is determined predominantly through the restricted expression of the α subunit of IL-5R (Takagi et al., 1995). The small percentage

(7%) of other αIL-5R mRNA positive cells from asthmatics could be basophils or B cells (Lopez et al., 1991).

Alternate splicing of the αIL-5R mRNA transcripts generates either a membrane-anchored or soluble isoform (Fig. 3) (Tavernier et al., 1992; Devos et al., 1993). The membrane-anchored form is attached to the surface of the cell membrane and combines with the β subunit, resulting in the high affinity αIL-5R. The membrane-anchored receptor complex triggers biological responses through specific intracellular signal transduction pathways (Fig. 2). In contrast to the membranous isoform, the secreted form of the α subunit is released from cells into biological fluids where it binds to IL-5 to inhibit IL-5 interaction with the membrane anchored isoform (Devos et al., 1993; Tavernier et al., 1992). Both soluble IL-5Rα isoform and membrane-anchored IL-5R complex bind IL-5 with equally high affinity (Devos et al., 1993). It has been previously reported that 90% of the manufactured αIL-5R mRNA may be in the soluble form and 10% in the membrane-anchored form (Tavernier et al., 1991; Devos et al., 1993). These observations also suggested that high levels of IL-5 may lead to the preferential expression of the soluble rather than the membranous form of the αIL-5R and that the soluble form may play a potential physiological role by attenuating the function of eosinophils in immune reactions. The direct correlation of αIL-5Rs mRNA with FEV_1 and the inverse correlation of αIL-5Rm mRNA with FEV_1 found in this study support these concepts. Low levels of αIL-5Rs mRNA may lead to low FEV_1 in asthmatics because of the reduced ability of αIL-5Rs to act as an endogenous inhibitor of eosinophils. Moreover, high levels of αIL-5Rm mRNA may lead to enhanced activation of eosinophils and thereby reduced FEV_1.

However, it may be speculated that the differential expression of both αIL-5R isoforms may be even greater in severe asthma than that observed in the relatively mild, stable asthmatics characterised in this study because increasing αIL-5Rs mRNA expression may be a compensatory mechanism against excessive eosinophil function. The lack of correlation between αIL-5R and PC_{20} reflects the fact that there was a narrow distribution of PC_{20} whereas there was a variable distribution of the αIL-5R mRNA in the asthmatic groups studied. These results suggest that αIL-5R mRNA correlates better with the degree of chronic airflow limitation rather than with bronchial reactivity. Indeed, further evidence that αIL-5Rs and airway responsiveness are not associated comes from another recent study which showed that αIL-5Rs blocked BAL eosinophilia but had only a small effect on airway reactivity in antigen challenged mice (Yamaguchi et al., 1994).

No difference was observed in the expression of αIL-5Rm and αIL-5Rs mRNA between atopic and non-atopic control subjects, indicating that atopy is not a function of altered αIL-5Rm and αIL-5Rs mRNA transcription. This contrasts with the results from the asthmatic subjects in which αIL-5Rm and αIL-5Rs mRNA transcription appear to be closely linked to the disease state. Therefore altered αIL-5Rm and αIL-5Rs mRNA transcription seems to be specific to asthma rather than non-specific to inflammatory diseases per se.

In order to verify that the localised αIL-5R mRNA transcripts are translated into receptor proteins, immunocytochemical studies would need to be performed. Messenger RNA is a midproduct between gene transcription and translation. The localisation of mRNA provides evidence of gene expression, but does not prove active synthesis of the relevant protein.

Unfortunately an antibody to the IL-5R is currently unavailable so it is not possible to detect this protein as yet. Furthermore, even if an antibody were available, it is virtually impossible to detect soluble receptors by using immunocytochemistry in tissue sections because they would be washed away during sample preparation. Resolving differences in the levels of the two isoforms of αIL-5R would thus not be possible. The detection of mRNA coding these receptors and the transient nature of the mRNA transcripts have certain advantages as well. Firstly, the signals for the two isoforms are both conveniently localised in the eosinophil. In addition, *in situ* hybridisation with specific, radiolabelled riboprobes complementary to the mRNA coding the various receptor subunits and isoforms is likely more sensitive and more specific than binding labelled antibody to the receptor protein. For example, the IL-5R shares some conformational homology with the other members of the class 1 cytokine receptor family whereas there is considerable heterogeneity between the mRNA transcripts of this family, which is what makes them distinguishable from each other (Cosman et al., 1990).

7.0 SUMMARY

Expression of αIL-5R mRNA in bronchial mucosal biopsies from asthmatic and control individuals was studied using the technique of *in situ* hybridisation. Bronchial biopsies were obtained from 19 asthmatics and 20 controls. ^{35}S-labelled riboprobe was prepared from an αIL-5R cDNA and hybridised to permeabilized tissue sections. Negative controls, including αIL-5R sense riboprobes and antisense probes after pre-treatment with RNase, were used in order to verify the specificity of the αIL-5R cDNA probes. Specific positive hybridisation for αIL-5R mRNA was demonstrated in the bronchial mucosa in asthmatics. Cells which exhibited hybridisation signals were those which are located beneath the epithelial basement membrane. In contrast, there was no or very few hybridisation in the non-asthmatic control groups. No hybridisation was observed in negative controls.

There was differential expression of αIL-5R mRNA between bronchial biopsies of asthmatics and controls. Asthmatic biopsies had increased expression of αIL-5R mRNA which was mainly co-localized to the eosinophils. When αIL-5R mRNA expression was compared with clinical features of airway obstruction, there was a significant direct correlation of αIL-5Rs mRNA with FEV_1 and a significant inverse correlation of αIL-5Rm mRNA with FEV_1. In addition, there was a significant inverse correlation between αIL-5Rm and αIL-5Rs mRNA. There was no correlation between the αIL-5R isoforms and methacholine PC_{20}.

8.0 CONCLUSION AND SUGGESTIONS FOR FUTURE STUDIES

This thesis has presented evidence for an increase in the number of αIL-5R mRNA positive cells in asthmatic individuals and there is a differential expression of membrane-anchored and soluble isoforms of αIL-5R mRNA, that number positive cells expressing αIL5R mRNA is associated with eosinophil count and that αIL5R mRNA expression correlates with the degree of airway obstruction rather than airway sensitivity. These results strongly support a role for IL-5 in the tissue eosinophilia in asthma and the need for deeper understanding of αIL-5R function in asthma.

One line of further study would be to examine the response of αIL-5R expression to antigen challenge and steroid treatment in asthmatics in order to strengthen the rationale that changes in αIL-5R isoform expression are central to asthma pathogenesis. Studying the expression of other cytokine receptors such as IL-4R and GM-CSFR in asthma would be also an interesting area to explore. Transcriptional and translational control of the αIL-5R is yet another line of investigation to be elucidated. Although transforming growth factor β1 (TGF β1) has already been demonstrated to downregulate αIL-5R mRNA expression (Zanders, 1994), the effect of cytokines on the generation of different mRNA splice products of αIL-5R has yet to be determined.

Moreover, αIL-5Rs may serve as αIL-5R antagonists of the membrane-anchored isoform. To explore this concept, investigation of αIL-5Rs as a potential therapeutic agent in eosinophil-associated diseases such as asthma is an important area of further study. This

innovative approach to treat asthma through the specific regulation and modulation of cytokines and their receptors might be more beneficial than current therapies because this would interfere early in the events leading to eosinophilia and the development of airway obstruction. Furthermore, the use of αIL5R receptor antagonists as specific anti-eosinophil therapeutics might have fewer side effects than steroids which are broad acting immunosuppressants.

Identifying IL-5 receptors in asthma might open up new options in improving the current understanding of the pathogenesis of asthma and might help to design new therapeutic approaches.

9.6 REFERENCES

Ackerman S.J. (1993). Characterization and functions of eosinophil granule proteins; in Makino S. Fukuda T. (eds): Eosinophils: Biological and Clinical Aspects. Boca Raton. CRC Press. pp 33-74.

American Thoracic Society. (1987). Standards for the diagnosis and the cure patients with chronic obstructive pulmonary disease (COPD) and asthma. Am. Rev. Respir. Dis. 136(1):225-244.

Askenase P.W. (1980). Immunopathology of parasitic diseases: Involvment of basophils and mast cells. Springer Semin. Immunopathol. 2:417-442

Assoian R.K., Fleurdelys B.E., Stevenson H.C., Miller P.J., Madtes D.K., Raines E.W., Ross R. and Sporn M. (1987). Expression and secretion type ß transforming growth factor by activated human macrophage. Proc. Natl. Acad. Sci. USA. 84:6020-6024.

Azuma C., Tanabe T., Konishi T., Noma T., Matsuda F., Yaoita Y., Takatsu K., Hammarstrom L., Smith C.I.E., Severinson E. and Honjo T. (1986). Cloning of cDNA for human T-cell replacing factor (interleukin-5) and comparison with the murine homologue. Nucleic Acid Res. 14:9149-9158.

Azzawi M., Bradley B., Jeffery P.K., Frew A.J., Wardlaw A.J., Knowles G., Assoufi B., Collins J.V., Durham S.R. and Kay A.B. (1990). Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. Am. Rev. Respir. Dis. 142:1407-1413.

Baggiolini M. and Dahinden C.A. (1994). CC chemokines in allergic inflammation. Immunol. Today. 15:127-133.

Bazan, J. F. (1990). Structural design and molecular evolution of a cytokine receptor superfamily. Proc. Natl. Acad. Sci. USA. 87:6934-6938.

Bazan, J.F. (1993). Emerging families of cytokines and receptors. Curr. Biol. 3:603-606.

Beasley R., Roche W.R., Roberts J.A. and Holgate S.T. (1989). Cellular events in the bronchi in mild asthma and after bronchial provocation. Am. Rev. Respir. Dis. 139:806-817.

Bentley A.M., Menz G., Storz C., Robinson D.S., Bradley B., Jeffery P.K., Durham S.R. and Kay A.B. (1992). Identification of T lymphocytes, macrophages and activated eosinophils in the bronchial mucosa in intrinsic asthma: relationship to symptoms and bronchial responsiveness. Am. Rev. Respir. Dis. 146:500-506.

Bentley A.M., Meng Q., Robinson D.S., Hamid Q., Kay A.B. and Durham S.R. (1993). Increases in activated T lymphocytes, eosinophils, and cytokine mRNA expression for interleukin-5 and granulocyte/macrophage colony-stimulating factor in bronchial biopsies after allergen inhalation challenge in atopic asthmatics. Am. J. Respir. Cell Mol. Biol. 8:35-42.

Bentley A.M., Hamid Q., Robinson S., Schotman E., Meng Q., Assofi B., Kay A.B. and Durham S.R. (1996). Prednisolone Treatment in Asthma: Reduction in the numbers of Eosinophils, T cells, Tryptase-only positive Mast cells, modulation of IL-4, IL-5, and Interferon-gamma cytokine gene expression within the bronchial mucosa. Am. J. Respir. Crit. Care Med. 153:551-556.

Bischoff S.C., Brunner T., De Weck A.L. and Dahinden C.A. (1990). Interleukin 5 modifies histamine release and leukotriene generation by human basophils in response to deverse agonists. J. Exp. Med. 172:1577-1582.

Bochner B.S., Luscinskas F.W., Gimbrone Jr. M.A., Newman W., Sterbinsky S.A., Derse-Anthony C.P., Klunk D. and Schleimer R.P. (1991). Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1-activated human vascular endothelial cells. contributions of endothelial cell adhesion molecules. J. Exp. Med. 173:1553-1556.

Boulay J.L. and Paul W.E. (1992a). The interleukin-4 family of lymphokines. Curr. Opin. Immunol. 4:294-298.

Boulay J.L. and Paul W.E. (1992b). The interleukin-4-related lymphokines and their binding to hematopoietin receptors. J. Biol. Chem. 267:20525-20528.

Boulay J.L. and Paul W.E. (1993). Hematopoietin-sub-families: classification based on size, gene organization, and secondary structures. Curr. Biol. 3:573-581.

Bousquet J., Chanez P., Lacoste J.Y., Barneon G., Ghavanian N., Enander J. Venge P., Ahlstedt S., Simony Lafontaine J., Godrad P. and Francois-Bernard M. (1990). Eosinophilic inflammation in asthma. N. Engl. J. Med. 323 (15): 1033-1039

Bradding P., Feather I.H., Wilson S., Bardin P.G., Heusser C.H. Holgate S.T. and Howarth P.H. (1993). Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. J. Immunol. 151:3853-3865.

Broide D.H., Paine M.M. and Firestein G.S. (1992) Eosinophils express interleukin-5 and granulocyte macrophage-colony stimulating factor mRNA at sites of allergic inflammation in asthmatics. J. Clin Invest 90:1414-1424

Campbell H.D., Tucker W.Q.J., Hort Y., Martinson M.E., Clutterbuck E.J., Sanderson C.J. and Young I.G. (1987). Molecular cloning and expression of the gene encoding human eosinophil differentiation factor (interleukin-5). Proc. Natl. Acad Sci. USA. 84:6629-6633.

Chand N., Harrison J.E., Rooney S., Pillar J., Jakubicki R., Nolan K., Diamantis W and Sofia R.D. (1992). Anti-IL-5 monoclonal antibody inhibits allergic late phase bronchial eosinophilia in guinea pigs: a therapeutic approach. Eur. J. Pharmacol. 211(1): 121-123.

Cher D.J. and Mosmann T.R. (1987). Two types of murine helper T cell clone II Delayed-type hypersensitivity is mediated by Th1 clones J. Immunol. 138:3688-3694.

Cherwinski H.M., Schumacher J.H., Brown K.D. and Mosmann T.R. (1987). Two types of mouse helper T cell clone. III: Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally mono-specific bioassays, and monoclonal antibodies. J. Exp. Med. 166:1229-1244

Clutterbuck E.J., Hirst E.M.A. and Sanderson C.J. (1989). Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6 and GM-CSF. Blood. 73:1504-1512.

Coca F. and Cook R.A. (1923). On the classification of the phenomena of hypersensitiveness. J. Immunol. 8:163.

Coffman R.L., Seymour B.W.P., Hudak S. Jackson J and Rennnick D. (1989). Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. Science 245: 308-310.

Collier P.J., Ramsey A.J., Austin P. and Gilbert P. (1990). Growth inhibitory and biocidal activity of some isothiazolone biocides. J. Appl. Bacteriol. 69: 569-577.

Cordell J.L., Falini B., Erber W.N., Ghosh A.K., Abdulaziz Z., Macdonald S., Pulford K.A.F., Stein H. and Mason D.Y. (1984) Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J. Histochem. Cytochem. 32:219-229.

Cornelis S., Plaetinck G., Devos R., Van der Heyden J., Tavernier J., Sanderson C.J., Guisez Y. and Fiers W. (1995a). Detailed analysis of the IL-5IL-5R alpha interaction: characterization of crucial residues on the ligand and the receptor. EMBO J. 14:3395-3402.

Cornelis S., Fache I., Van der Heyden J., Guisez Y., Tavernier J., Devos R., Fiers W. and Plaetinck G. (1995b). Characterization of critical residues in the cytoplasmic domain of the human interleukin-5 receptor α chain required for growth signal transduction. Eur. J. Immunol. 25:1857-1864.

Corrigan C.J., Hamid Q., North J., Barkans J., Moqbel R., Durham S., Gemou-Engesaeth V. and Kay A.B. (1995). Peripheral blood CD4 but not CD8 T-lymphocytes in patients with exacerbation of asthma transcribe and translate messenger RNA encoding cytokines which prolong eosinophil survival in the context of a Th2-type pattern: Effect of glucocorticoid therapy. Am. J. Respir. Cell Mol. Biol. 12:567-578.

Cosman D., Lyman S.D., Idzerda R.L., Beckman M.P., Park L.S., Goodwin R.G. and March C.J. (1990). A new cytokine receptor superfamily. Trends Biochem. Sci. 15:265-270.

Costa J.J., Matossian K., Resnick M.B., Beil W., Wong D.T.W., Gordon J.R., Dorak A.M., Weller P.F. and Galli S.J. (1993). Human eosinophils can express the cytokines tumor necrosis factor-α and macrophage inflammatory protein-1α. J. Clin. Invest. 91:2673-2684.

Cox K.H., Delon D.V., Angerer L.M. and Angerer R.C. (1984). Detection of mRNAs in sea urchin embryos by in situ hybridization using asymmetric RNA probes. Dev. Biol. 101:485-502.

Cutz E., Levison H. and Cooper D.M. (1978). Ultrastructure of airways in children with asthma. Histopathol. 2:407-421.

Dent L.A., Strath M., Mellor A.L. and Sanderson C.J. (1990). Eosinophilia in transgenic mice expressing interleukin-5. J. Exp. Med. 172:1425-1431.

Devos R., Plaetinck G., Van der Heyden J., Cornelis S., Vandekerckhove J., Fiers W. and Tavernier J. (1991). Molecular basis of a high affinity murine interleukin 5 receptor. EMBO J. 10: 2133-2137.

Devos R., Y. Guisez Y., Cornelis S., Verhee A., Van der Heyden J., Manneberg M., Lahm H.W., Fiers W., Tavernier J. and Plaetnick G. (1993). Recombinant soluble human interleukin-5 (hIL-5) receptor molecules: cross-linking and stoichiometry of binding to IL-5. J. Biol. Chem. 268: 6581-6587.

Devos R., Guisez Y., Plaetinck G., Cornelis S., Tavernier J., Van der Heyden J., Foley L.H. and Scheffler J.E. (1994). Covalent modification of the interleukin-5 receptor by isothiazolones leads to inhibition of the binding of interleukin-5. Eur. J. Biochem. 255: 635-640.

Dubucquoi S., Desreumaux P., Janin A., Klein O., Goldman M., Tavernier J., Capron A. and Capron M. (1994). Interleukin 5 synthesis by eosinophils: association with granules and immunoglobulin-dependent secretion. J. Exp. Med. 179:703-708.

Dunnill M.S., Massarella G.R. and Anderson J.A. (1969). A comparison of the quantitative anatomy of the bronchi in normal subjects, in status asthmaticus, in chronic bronchitis, and in emphysema. Thorax. 24:176-179.

Durham S.R. and Kay A.B. (1985). Eosinophils, bronchial hyperreactivity and late-phase asthmatic reactions. Clin. Allergy. 15:411-418

Durham S.R., Sun Ying., Varney V.A., Jacobson M.R., Sudderick R.M., Mackay I.S., Kay A.B. and Hamid Q. (1992). Cytokine messenger RNA expression for IL-3, IL-4, IL-5 and granulocyte/macrophage colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. J. Immunol. 148:2390-2394.

Dvorak A.M., Ackerman S.J. and Weller P.F. (1991). Subcellular morphology and biochemistry of eosinophils; in Harris J.R. (ed): Megakaryocytes, platelets, macrophages, and eosinophils. Blood Cell Biochemistry. London, Plenum Publishing. Vol. 2:237-344.

Enright Y., Chua S. and Lim D.T. (1989). Pulmonary eosinophilic syndromes. Ann. Allergy. 62:277-283.

Fernandez-Botran R. (1991). Soluble cytokine receptors: their role in immunoregulation. FASEB J. 5:2567-2574.

Filley W.V., Holley K.E., Kephart G.M. and Gleich G.J. (1982). Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. Lancet. 2:11-16.

Fiorentino D.F., Bond M.W. and Mosmann T.R. (1989). Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J. Exp. Med. 170:2081-2095.

Foxwell B.M.J., Barett K. and Feldmann M. (1992). Cytokine receptors: Structure and signal transduction. Clin. Exp. Immunol. 90:161-169.

Frew A.J. and Kay A.B. (1988). The relationship between infiltrating CD4+ lymphocytes, activated eosinophils, and the magnitude of the allergen-induced late phase cutaneous reaction in man. J. Immunol. 141:4158-4164

Frick W.E., Sedgwick J.B. and Busse W.W. (1989). The appearance of hypodense eosinophils in antige-dependent late phase asthma. Am. Rev Respir. Dis. 139.1401-1406.

Fujisawa T., Abu-Ghazaleh R., Kita H., Sanderson C.J. and Gleich G.J. (1990). Regulatory effect of cytokines on eosinophil degranulation. J. Immunol. 144:642-646.

Gall J.G. and Pardue M.L. (1969). Formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proc. Natl. Acad. Sci. U.S.A. 63:378-383.

Galli S.J. (1993). New concepts about the mast cells. New Engl. J. Med. 328:257-265

Gerblich A.A., Campbell A.E. and Schuyler M.R. (1984). Changes in T-lymphocyte subpopulations after antigenic bronchial provocation in asthma. N. Engl. J. Med. 310:1349-1352.

Gerblich A.A., Salik H. and Schuyler M.R. (1991). Dynamic T-cell changes in peripheral blood and bronchoalveolar lavage after antigen bronchoprovocation in asthmatics. Am. Rev. Respir. Dis. 143:533-537.

Gillis S. (1991). Cytokine receptors. Curr. Opin. Immunol. 3:315-319.

Glantz S.A. (1992). Primer of Biostatistics. McGraw-Hill, Inc. (3rd ed.).

Gleich G.J., Frigas E., Loegering D.A., Wassom D.L. and Steinmuller D. (1979). Cytotoxic properties of the eosinophil major basic protein. J. Immunol. 123:2925-2927.

Gonzalez M.C., Diaz P., Galleguillos F.R., Ancic P., Cromwell O. and Kay A.B. (1987). Allergen-induced recruitment of bronchoalveolar helper (OKT4) and suppressor (OKT8) T-cells in asthma. Relative increases in OKT8 in single early responders compared with those in late-phase responders. Am. Rev. Respir. Dis. 136:600-604.

Gorman D.M., Itoh N., Jenkins N.A., Gilbert D.J., Copeland N.G. and Miyajima A. (1992). Chromosomal localization and organization of the murine genes encoding the beta subunits (AIC2A and AIC2B) of the interleukin 3, granulocyte/macrophage colony-stimulating factor, and interleukin 5 receptors. J. Biol. Chem. 267:15842-15848.

Gulbenkian A.R., Egan R.W., Fernandez X., Jones H., Kreutner W., Kung T., Payvandi F., Sullivan L., Zurcher J.A. and Watnik A.S. (1992). Interleukin-5 modulates eosinophil accumulation in allergic guinea pig lung. Am. Rev. Resp. Dis. 146: 263-265.

Gundel R.H., Letts G. and Gleich G.J. (1991). Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. J. Clin Invest 87:1470-1473

Guo C.B , Liu M.C., Galli S.J., Kagey Sobotka A. and Lichtenstein L.M (1990) The histamine containig cells in the late phase response in the lung are basophils. J. Allergy Clin Immunol. 85:A113.

Hamaguchi Y., Kanakura Y., Fujita J., Takeda S-I., Nakano T., Tarui S , Honjo T. and Kitamura Y. (1987). Interleukin 4 as an essential factor for in vitro clonal growth of murine connective tissue-type mast cells. J. Exp. Med. 165:268-273.

Hamid Q., Azzawi M., Sun Ying., Moqbel R., Wardlaw A.J., Corrigan C J., Bradley B., Durham S.R., Collins J.V., Jeffery P.K., Quint D.J. and Kay A B. (1991). Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. J Clin. Invest. 87:1541-1546.

Hamid Q., Boguniewicz M. and Leung D Y. (1994). Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. J. Clin. Invest. 94:870-6.

Hayashida K., Kiatmura T., Gorman D.M., Arai K., Yokota T. and Miyajima A. (1990). Molecular cloning of a second subunit of the human GM-CSF receptor Reconstitution of a high affinity GM-CSF receptor. Proc. Natl. Acad. Sci. USA 87:9655-9659.

Hitoshi Y., Yamaguchi N., Korenaga M., Mita M., Tominaga A., and Takatsu K. (1991). In vivo administration of antibody to murine IL-5 receptor inhibits eosinophilia of IL-5 transgenic mice. Int. Immunol. 3: 135-139.

Horn B.R., Rbin E.D., Theodero J. and Van Kessel A. (1975). Total eosinophil counts in the management of bronchial asthma. N. Engl. J. Med. 292:1152-1155.

Ihle J.N. and Kerr I.M. (1995). Jaks and Stats in signaling by the cytokine receptor superfamily. Trends Genet. 11:69-74.

Imamura F., Takaki S., Akagi K., Ando M., Yamamura K.I., Takatsu K. and Tominaga A. (1994). The murine interleukin-5 receptor alpha-subunit gene: Characterization of the gene structure and chromosome mapping. DNA Cell Biol. 13(3):283-293.

Ishizaka K. and Ishizaka T. (1971). Mechanism of reagenic hypersensitivity-a review. Clin. Allergy 1:9-24.

Isobe M., Kumura Y , Murata Y , Takaki S., Tominaga A., Takatsu K. and Ogita Z. (1992). Localization of the gene encoding the α subunit of human interleukin-5 receptor (IL5RA) to chromosome region 3p24-3p26. Genomics 14:744

Iwasaki, K., Torisu, M. and Fujimura, T. (1986). Malignant tumor and eosinophils I. Prognostic significance in gastric cancer. Cancer 58:1321-1327.

Jeffery P.K., Wardlaw A.J., Nelson F.C., Collins J.V. and Kay A.B. (1989). Bronchial biopsies in asthma. An ultrastructural, quantitative study and correlation with hyperreactivity. Am. Rev. Respir. Dis. 140:1745-1753.

Johnston Jr. R.B. (1988). Monocytes and macrophages. N. Engl. J. Med. 318:747-752.

Johnston S.L. and Holgate S.T. (1991). The inflammatory response in asthma. Brit J. Hosp. Med. 46:84-90.

Joseph M., Tonnel A.B., Torpier G. and Capron A. (1983). Involvement of immunoglobulin E in the secretory processes of alveolar macrophages from asthmatic patients. J. Clin. Invest. 71:221-230.

Kameyoshi Y., Dorschner A., Mallet A.I., Christophers E. and Schroder J.M. (1992). Cytokine RANTES released by thrombin-stimulated platelets is a potent attractant for human eosinophils. J. Exp. Med. 176:587-592.

Kay A.B., Ying S., Varney V., Durham S.R., Moqbel R., Wardlaw A.J. and Hamid Q. (1991). Messenger RNA expression of the cytokine gene cluster, IL-3, IL-4, IL-5, and GM-CSF, in allergen-induced late-phase reactions in atopic subjects. J. Exp. Med. 173:775-778.

Kay A.B. (1992). "Helper" (CD4+) T cells and eosinophils in allergy and asthma. Am. Rev. Respir. Dis. 145:S22-S26.

Kay A.B. (1994). Origin of type 2 helper T cells. New Engl. J. Med. 330:567-569.

Kimani G., Tonnesen M.G. and Henson P.M. (1988). Stimulation of eosinophil adherence to human vascular endothelial cells in vitro by platelet-activating factor. J. Immunol. 140:3161-3166.

Kinashi T., Harada N., Severinson E., Tanabe T., Sideras P., Konishi M., Azuma C., Tominaga A., Bergstedt-Lindevist S., Takahashi M., Matsuda F., Yaoita Y., Takatsu K. and Honjo T. (1986). Cloning of complementory DNA encoding T-cell replacing factor and identity with B-cell growth factor II. Nature. 324:70-73.

Kinet J.P. (1989). The high-affinity receptor for IgE. Curr Opin Immunol 2:499-505.

Kita H., Oshimishi T., Okubo Y., Weiler D., Abrams J.S. and Gleich G.J. (1991) Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. J. Exp. Med. 174:745-748

Kitamura T., Tange T., Terasawa T., Chiba S., Kuwaki T., Miyagawa K., Piao Y., Miyazono K., Urabe A. and Takaku F. (1989). Establishment and characterization of a unique human cell line that proliferates dependently on GM-CSF, IL-3, or erythropoietin. J Cell Physiol. 140:323-334.

Koshino T., Teshima S., Fukushima N., Takaishi T., Hirai K., Miyamoto Y., Arai Y., Sano Y., Ito K. and Morita Y. (1993). Identification of basophils by immunocytochemistry in the airways of post-mortem cases of fatal asthma. Clin. Exp. Allergy 23:919-925.

Laitinen L.A., Laitinen A. and Haahtela T. (1993). Airway mucosal inflammation even in patients with newly diagnosed asthma. Am. Rev. Respir. Dis. 147:697-704.

Lee T.C., Lenihan D.J., Malone B., Roddy L.L. and Wasserman S.I. (1984). Increased biosynthesis of platelet-activating factor in activated human eosinophils J Biol Chem. 259:5526-5530.

Leung D.Y.M., Martin R.J., Szefler S.J., Sher E.R., Ying S., Kay A.B and Hamid Q. (1995). Dysregulation of Interleukin-5, and Interferon γ gene expression in steroid-resistant astma. J. Exp. Med. 181:33-40.

Limaye A.P., Abrams J.S., Silver J.E., Ottesen E.A. and Nutman T.B. (1990). Regulation of parasite-induced eosinophilia: Selectively increased interleukin 5 production in helminth-infected patients. J. Exp. Med. 172: 399-402.

Liu M.C., Bleecker E.R., Lichtenstein L M., Kagey-Sobatka A., Niv Y., Mclemore T.L., Permutt S., Proud D. and Hubbard W.C. (1990). Evidence for elevated levels of histamine, prostaglandin D2 and other bronchoconstricting prostaglandins in the airways of subjects with mild asthma. Am. Rev. Respir. Dis. 142:126-132.

Liu M.C., Hubbard W.C., Proud D., Stealey B.A., Galli S.J., Kagey-Sobotka A., Bleecker E.R. and Lichtenstein L.M. (1991). Immediate and late inflammatory responses to ragweed antigen challenge of the peripheral airways in allergic asthmatics. Am. Rev. Respir. Dis. 144:51-58.

Lopez A F , Sanderson C.J , Gamble J. R., Campbell H D., Young I.J. and Vadas M A (1988) Recombinant human interleukin 5 is a selective activator of human eosinophil function. J. Exp Med. 167:219-224.

Lopez A.F., Eglinton J M., Lyons A.B., Tapley P.M., To L.B., Park L.S., Clarke S.C and Vadas M.A. (1990). Human interleukin-3 inhibits the binding of granulocyte-macrophage colony-stimulating factor and interleukin-5 to basophils and strongly enhances their functional activity. J. Cell Physiol. 145:69-77.

Lopez A.F., Vadas M.V., Woodcock J.M., Milton S.E., Lewis A., Elliott M.J., Gillis D , Ireland R., Olwell E. and Park L.S. (1991). Interleukin-5, interleukin-3, and granulocyte-macrophage colony-stimulating factor cross-compete for binding to cell surface receptors on human eosinophils J Biol. Chem. 266:24741-24747.

Macdonald D., Gordon A.A., Kajitani H., Enokihara H. and Barrett A.J. (1990). Interleukin-2 treatment-associated eosinophilia is mediated by interleukin-5 production. Br. J. Haematol. 76:168-173.

McFadden E.R.Jr. and Gilbert I.A. (1992). Asthma. New Engl. J. Med. 327:1928-1937.

Metzger W.J., Richerson H.B., Worden K., Monick M. and Hunninghake G.W. (1986). Bronchoalveolar lavage of allergic asthmatic patients following allergen bronchoprovocation. Chest. 89:477-483.

Migita M., Yamagushi N., Mita S., Higuchi S., Hitoshi Y., Yoshida Y., Tomonaga M., Matsuda I., Tominaga A. and Takatsu K. (1991). Characterization of the human IL-5 receptors on eosinophils. Cell. Immunol. 133: 484-497.

Milburn M.V., Hassel A.M., Lambert M.H, Jordan S.R., Proudfoot A.E., Graber P. and Wells T.N.C. (1993). A novel dimer configuration revealed by the crystal structure at 2.4 °A resolution of human interleukin-5. Nature 363: 172-176.

Minamitake Y., Kodama S., Katayama T., Adachi H., Tanaka S. and Tsujimoto M. (1990). Structure of recombinant human interleukin 5 produced by Chinese hamster ovary cells. J. Biochem. 107:292-297.

Miyajima A., Mui A.L.-F., Ogorochi T. and Sakamaki K. (1993). Receptors for granulocyte-macrophage colony-stimulating factor, interleukin-3, and interleukin-5. Blood. 82:1960-1974.

Miyazaki T., Maruyama M., Yamada G., Hatakeyama M. and Tanigushi T. (1991). The integrity of the conserved 'WS motif' common to the IL-2 and other cytokine receptors is essential for ligand binding and signal transduction. EMBO J. 10:3191-3197.

Moqbel R., Hamid Q., Ying S., Barkans J., Hartnell A., Tsicopoulos A., Wardlaw A.J. and Kay A.B. (1991). Expression of mRNA and immunoreactivity for the granulocyte/macrophage colony-stimulating factor in activated human eosinophils. J. Exp. Med. 174:749-752.

Mosmann T.R., Cherwinski H., Bond M.W., Giedlin M.A. and Coffman R.L. (1986). Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. 136:2348-2357.

Mosmann T.R. and Coffman R.L. (1989). Th1 and Th2 cells: different patterns of lymphokine secretion to different functional properties. Annu. Rev. Immunol. 7:145-173.

Motojima S., Frigas E., Loegering D.A. and Gleich G.J. (1989). Toxicity of eosinophil cationic proteins for guinea pig tracheal epithelium in vitro. Am. Rev. Respir. Dis. 139:801-805.

Motojima S., Akutsu I., Fukuda T., Makino S. and Takatus K. (1993). Clinical significance of measuring levels of sputum and serum ECP and serum IL-5 in bronchial asthma. Allergy 48:98-106.

Mui A.L. and Miyajima A. (1994). Interleukin-3 and granulocyte-macrophage colony-stimulating factor receptor signal transduction. Proc. Soc. Exp. Biol. Med. 206(3):284-288.

Murata Y., Takaki S., Migita M., Kikuchi Y., Tominaga A. and Takatsu K. (1992). Molecular cloning and expression of the human interleukin-5 receptor. J. Exp. Med. 175:341-351.

Nicola, N..A.(1989). Hemopoietic cell growth factors and their receptors. Annu. Rev. Biochem. 58:45-77.

O'Byrne P.M., Dolovich J. and Hargreave F.E. (1987). Late asthmatic responses. Am. Rev. Respir. Dis. 136:740-751.

Ohnishi T., Kita H., Weiler D., Sur S., Sedgwick J.B., Calhoun W.J., Busse W.W., Abrams J.S. and Gleich G.J. (1993a). IL-5 is the predominant eosinophil-active cytokine in the antigen-induced pulmonary late-phase rection. Am. Rev. Respir. Dis. 147:901-907.

Ohnishi T., Sur S., Collins D.S., Fish J.E., Gleich G.J. and Peters S.P. (1993b). Eosinophil survival activity identified as interleukin-5 is associated with eosinophil recruitment and degranulation and lung injury twenty-four hours after segmental antigen lung challenge. J. Allergy Clin. Immunol. 92:607-615.

Owen W F , Rothenberg M.E., Petersen J., Weller P.F., Silberstein D., Sheffer A.L., Stevens R.L , Soberman R.J., and Austen K.F. (1989). Interleukin 5 and phenotypically altered eosinophils in the blood of patients with the idiophatic hypereosinophilic syndrome. J. Exp. Med. 170:343-348.

Pazdrak K., Schreiber D., Forsythe P., Justement L., Alam R. (1995). The intracellular signal transduction mechanism of interleukin 5 in eosinophils: the involvment of lyn tyrosine kinase and the Ras-Raf-1-MEK-microtuble-associated protein kinase pathway. J. Exp. Med. 181(5):1827-34

Pene J., Rousset F., Briere F., Chretien I., Wademan J., Bonnefory J.Y. and de Vries J.E. (1988). Interleukin-5 enhances IL-4-induced IgE production by normal human B cells: the role of soluble CD23 antigen. Eur. J. Immunol. 18: 929-935.

Plaetinck G., Van der Heyden, Tavernier J., Fache I., Tuypens T., Fischkoff S., Fiers W. and Devos R. (1990). Characterization of interleukin 5 receptors on eosinophilic sublines from human promyelocytic leukemia (HL-60) cells. J. Exp. Med. 172: 683-691.

Plaut M. (1990). Antigen specific lymphokine secretory patterns in atopic disease. J. Immunol. 144:4497-4500.

Pretlow T.P., Keither E.F., Cryar A.K., Bartolucci A.A., Pitts A.M.,Pretlow II T.G.,Kimball P.M. and Boohaker E.A. (1983). Eosinophil infilitration of human colonic carcinomas as a prognostic indicator. Cancer Res. 43: 2997-3000.

Pretolani M., Ruffie C., Lapae Silva JR., Joseph D., Lopp R.R. and Vargaftig BB. (1994). Antibody to very late activation antigen prevents antigen-induced bronchial hyperreactivity and cellular infilitration in the guinea pig airways. J. Exp. Med. 180: 795-805.

Prin L., Capron M., Tonnel A.B., Blentry O. And Capron A. (1983). Heterogeneity of human periheral blood eosinophils: Variability in cell density and cytotoxic ability in relation to the level and origin of hypereosinophilia. Int. Arch. Allergy Appl. Immunol. 72:336-346.

Purkerson J.M. and Isakson P.C. (1992). Interleukin 5 (IL-5) provides a signal that is required in addition to IL-4 for isotype switching to immunoglobulin (Ig) G1 and IgE. J. Exp. Med. 175:973-982.

Rak S., Lowhagen O. and Venge P. (1988). The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen-allergic patients. J. Allergy Clin. Immunol. 82:470-480.

Resnick M.B. and Weller P.F. (1993). Mechanisms of eosinophil recruitment. Am. J. Respir. Cell Mol. Biol. 8:349-355.

Robbins S.L. and Kumar V (1987). Basic Pathology, 4th Edition Toronto, W B Saunders Company.

Robinson D.S., Hamid Q , Sun Ying., Tsicopoulos A., Barkans J., Bentley A M , Corrigan C., Durham S.R. and Kay A.B. (1992). Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. N. Engl. J. Med. 326:298-304.

Robinson D.S., Bentley A.M., Hartnell A., Kay B. and Durham S.R. (1993a) Activated memory T helper cells in bronchoalveolar lavage from atopic asthmatics Relationship to asthma symptoms, lung function and bronchial responsiveness. Thorax 48:26-32.

Robinson D., Hamid Q., Ying S., Bentley A., Assoufi B., Durham S. and Kay A.B.(1993b). Prednisolone treatment in asthma is associated with modulation of bronchoalveolar lavage cell interleukin-4, interleukin-5, and interferon-γ cytokine gene expression. Am. Rev. Respir. Dis. 148(2):401-406.

Romagnani S. (1992). Induction of Th1 and Th2 responses: a key role for the "natural" immune response? Immunol. Today 13:379-381.

Rossi G.A., Crimi E., Lantero S., Gianiorio P., Oddera S., Crimi P. and Brusasco V. (1991). Late-phase asthmatic reaction to inhaled allergen is associated with early recruitment of eosinophils in the airways. Am. Rev. Respir. Dis. 144: 379-383.

Romagnani S. (1990). Regulation and degranulation of human IgE synthesis. Immunology Today. 11:316-321.

Romagnani S. (1992). Human Th1 and Th2 doubt no more. Immunol. Today. 12:256-257.

Sakamaki K., Miyajima I., Kitamura T. and Miyajima A. (1992). Critical cytoplasmic domains of the common β subunit of the human GM-CSF, IL-3 and IL-5 receptors for growth signal transduction and tyrosine phosphorylation. EMBO J. 11:3541-3649.

Schimple A. and Wecker E. (1972). Replacement of T cell function by a T cell product. Nature 237:15-17.

Sehmi R., Wardlaw A.J., Cromwell O., Kurihara K., Waltmann P. and Kay A.B. (1992). Interleukin-5 selectively enhances the chemotactic response of eosinophils obtained from normal but not eosinophilic subjects. Blood. 79:2952-2959.

Shaw R.J., Walsh G M., Cromwell O., Moqbel R., Spry C.J.F. and Kay A.B. (1985). Activated human eosinophils generate SRS-A leukotrienes following IgG-dependent stimulation. Nature 316:150-152.

Shen Y., Baker E., Callen D.F., Sutherland G.R., Willson T.A., Raker S. and Gough N.M. (1992). Localization of the human GM-csf receptor β chain gene (CSF2RB) to chromosome 22q12.2→q13.1. Cytogenet Cell Genet. 61:175-177.

Spiteri M. A. and Poulter L.W. (1991). Characterization of immune inducer and supressor macrophages from normal human lung. Clin. Exp. Immunol. 83:157-162.

Stern M., Meagher L., Savill J. and Haslett C. (1992). Apoptosis in human eosinophils. Programmed cell death in the eosinophil leads to phagocytosis by macrophages and is modulated by IL-5. J. Immunol. 148:3543-3549.

Street N.E. and Mosmann T.R. (1991). Functional diversity of T-lymphocytes due to secretion of different cytokine patterns. FASEB J. 5:171-177.

Sun Z., Yergeau D.A., Tuypens T., Tavernier J., Paul C.C., Baumann M.A., Tenen D.G., and Ackerman S.J. (1995). Identification and characterization of a functional promoter region in the human eosinophil IL-5 receptor α subunit gene. J. Biol. Chem. 270:1462-1471.

Takafuji S., Bischoff S.C., de Weck A.L. and Dahinden C.A. (1991). IL-3 and IL-5 prime normal human eosinophils to produce leukotriene C4 in response to soluble agonists. J. Immunol. 147:3855-3861.

Takagi M., Hara T., Ichihara M., Takatsu K., and Miyajima A. (1995). Multi-colony stimulating activity of interleukin-5 (IL-5) on hematopoietic progenitors from transgenic mice that express IL-5 receptor α subunit constitutively. J. Exp. Med. 181:889-899.

Takaki S., Tominaga A., Hitoshi Y., Mita S., Sonoda E., Yamaguchi N., and Takatsu K. (1990). Molecular cloning and expression of the murine interleukin-5 receptor. EMBO J. 9:4367-4374.

Takaki S., Mita S., Kitamura T., Yonehara S., Yamaguchi N., Tominaga A., Miyajima A. and Takatsu K. (1991). Identification of the second subunit of the murine interleukin-5 receptor: interleukin-3 receptor-like protein. AIC2B, is a component of the high-affinity interleukin-5 receptor. EMBO J. 10:2833-2838.

Takaki, S., Murata, Y., Kitamura, T., Miyajima, A., Tominaga, A. and Takatsu, K. (1993). Reconstitution of the functional receptors for murine and human interleukin 5. J. Exp. Med. 177:1523-1529.

Tanimoto Y., Takahashi K. and Kimura I. (1992). Effects of cytokines on human basophil chemotaxis. Clin. Exp. Allergy. 22:1020-1025.

Tavernier J., Devos R., Cornelis S., Tuypens T., Van der Heyden J., Fiers W. and Plaetinck G. (1991). A human high affinity interleukin-5 receptor (IL-5) is composed of an IL-5-specific α chain and β chain shared with the receptor for GM-CSF. Cell. 66:1175-1184.

Tavernier J., Tuypens T., Plaetinck G., Verhee A,. Fiers W. and Devos R. (1992). Molecular basis of the membrane-anchored and two soluble isoforms of the human interleukin 5 receptor subunit. Proc. Natl. Acad. Sci. USA 89:7041-7045.

Tavernier J., Tuypens T., Verhee A., Plaetinck G., Devos R., Van der Hayden J., Guisez Y. and Oefner C. (1995). Identification of receptor- binding domains on human interleukin 5 and design of an interleukin 5-derived receptor antagonist. Proc. Natl. Acad. Sci. USA 92:5194-5198.

Thepen T., van Rooijen N. and Kraal G. (1989). Alveolar macrophage elimination in vivo is associated with an increase in pulmonary immune response in mice. J. Exp. Med. 170:499-509.

Tuypens T., Plaetinck, G., Baker E., Sutherland G., Brusselle G., Fiers W., Devos R. and Tavernier J. (1992). Organization and chromosomal localization of the human interleukin 5 receptor α -chain gene. Eur. Cytokine Netw. 3:451-459.

Valent P., Schmidt G., Besemer J., Mayer P., Zenke G., Liehl E., Hinterberger W., Lechner K., Maurer D. and Bettelheim P. (1989). Interleukin-3 is a differentiation factor for human basophils. Blood. 73:1763-1769.

van der Bruggen T., Caldehoven E., Kanters D., Coffer P., Raaijmakers J.A.M., Lammers J.W.J. and Koenderman L. (1995). Interleukin-5 signalling in human eosinophils involves JAK2 tyrosine kinase and Stat1α. Blood 85:1442-1448.

van Leeuwen B.H., Martinson M.E., Webb G.C. and Young J.G. (1989). Molecular organization of the cytokine gene cluster, involving the human IL-3, IL-4, IL-5, and GM-CSF genes on human chromosome 5. Blood. 73:1142-1148.

van Oosterhout A.J.M., Ladenius A.R.C., Savelkoul H.F.J., van Ark I., Delsman K.C. and Nijkamp F.P. (1993a). Effect of anti-IL-5 and IL-5 on airway hyperreactivity and eosinophils in guinea pigs. Am. Rev. Respir. Dis. 147:548-552.

van Oosterhout A.J M., Van Ark I., Hofman G., Savelkoul H.F.J. and Nijkamp F.P. (1993b). Recombinant interleukin-5 induces in vivo airway hyperresponsiveness to histamine in guinea pigs. Eur. J Pharmacol. 236:379-383.

Walker C., Kaegi M.K., Braun P. and Blaser K. (1991a). Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. J. Allergy Clin. Immunol. 88:935-942.

Walker C., Virchow J.-C.Jr., Bruijnzeel P.L.B. and Blaser K. (1991b). T cell subsets and their soluble products regulate eosinophilia in allergic and nonallergic asthma. J. Immunol. 146:1829-1835.

Walker C., Bode E., Boer L., Hansel T.T., Blaser K. and Virchow J.-C. Jr. (1992). Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. Am. Rev. Respir. Dis. 146:109-115.

Walker, C. (1993). The immunology of extrinsic and intrinsic asthma. New drugs in allergy and asthma. 97-106.

Walsh G.M., Hartnell A., Wardlaw A.J., Kurihara K., Sanderson C.J. and Kay. A.B. (1990). IL-5 enhances the in vitro adhesion of human eosinophils, but not neutrophils, in a leukocyte integrin (CD11/18)-dependent manner. Immunology. 71:258-265.

Walsh G.M., Mermod J.J., Hartnell A, Kay A.B. and Wardlaw A.J. (1991a). Human eosinophil, but not neutrophil, adherence to IL-1 stimulated human umbilical vascular endothelial cells is VLA-4 (very late antigen-4) dependent. J. Immunol. 146:3419-3423.

Walsh L.J., Trinchieri G., Waldorf H.A., Whittaker D. and Murphy G.F. (1991b). Human mast cells contain and release tumor necrosis factor alpha which induces endothelial leucocyte adhesion molecule-1. Proc. Natl. Acad. Sci. USA 88:4220-4223.

Wang J.M., Rambaldi A., Biondi A., Chen Z-G., Sanderson C.J. and Mantorani A. (1989). Recombianat human interleukin-5 is a selective eosinophil chemoattractant. Eur. J. Immunol. 19:701-705.

Wardlaw A.J., Dunnette S., Gleich G.J., Collins J.V. and Kay A.B. (1988). Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperreactivity. Am. Rev. Respir. Dis. 137:62-69.

Wardlaw A.J., Moqbel R., Cromwell O. and Kay A.B. (1986). Platelet-activating factor: a potent chemotactic and chemokinetic factor for human eosinophils. J. Clin. Invest. 78:1701-1706.

Watson S.P and James W. (1989). PCR and the cloning of receptor subtype genes. Trends in Pharmacol. Sci. 10:346-348.

Weller P.F. (1984). Eosinophilia. J. Allergy Clin. Immunol. 73:1-10.

Wenzel S.E., Fowler A.A.3d and Schwartz L.B. (1988). Activation of pulmonary mast cells by bronchoalveolar allergen challenge. In vivo release of histamine and tryptase in atopic subjects with and without asthma. Am. Rev. Respir. Dis. 137:1002-1008.

White M.V. and Kaliner M.A. (1991). Mast cells and asthma. Lung Biology in health and disease. 49:409-440.

Winquist I., Oloffson T., Olsson I., Persson A.M. and Hallberg T. (1982). Altered density, metabolism and surface receptors of eosinophils in eosinophilia. Immunology 47:531-539.

Wong D.T.W., Elovic A., Matossian K., Nagura N., McBride J., Chou M.Y., Gordon J.R., Rand T.H., Galli S.J. and Weller P.F. (1991). Eosinophils from patients with blood eosinophilia express transforming growth factor β1. Blood. 78:2702-2707.

Woolcock A.J., Peat J.K., Salome C.M., Yan K., Anderson S.D., Schoeffel R.E., McCowage G. and Killalea T. (1987). Prevalence of bronchial hyperresponsiveness and asthma in a rural adult population. Thorax 42:361 368.

Yamaguchi Y., Suda T., Suda J., Eguchi M., Miura Y., Harada N., Tominaga A. and Takatsu K. (1988a). Purified interleukin 5 supports the terminal differentiation and proliferation of murine eosinophilic precursors. J. Exp. Med. 167:43-56.

Yamaguchi Y., Hayashi Y., Sugama Y., Miura Y., Kasahara T., Kitamura S., Torisu M., Mita S., Tominaga A., Takatsu K. and Suda T. (1988b). Highly purified murine interleukin-5 (IL-5) stimulates eosinophil function and prolongs in vitro survival. J. Exp. Med. 167:1737-1742.

Yamaguchi Y., Matsui T., Kasahara T., Etott S., Tominaga A., Takatsu K., Miura Y. and Suda T. (1990). In vivo changes of hematopoietic progenitors and the expression of the interleukin 5 gene in eosinophilic mice infected with Toxocara canis. Exp. Hematol. 18: 1152-1157.

Yamaguchi Y., Suda T., Ohta S., Tominaga K., Miura Y. and Kasahara T. (1991). Analysis of the survival of mature human eosinophils: interleukin-5 prevents apoptosis in mature human eosinophils. Blood 178:2542-2547.

Yamaguchi S., Nagai H., Tanaka H., Tsujimoto M. and N. Tsuruoka. (1994). Time course study for antigen induce airway hyperreactivity and the effect of soluble IL-5 receptor. Life Sciences 54:472-475.

Yarden Y. and Ullrich A. (1988). Growth factor receptor tyrosine kinases. Ann. Rev. Biochem. 57:443-478.

Yen J., Hsieh Y.C.,Yen C.L., Chang C.C., Lin S. and Yen H.F.Y. (1995). Restoring the apoptosis suppression response to IL-5 confers on erythroleukemic cells a phenotype of IL-5-dependent growth. J. Immunol. 154:2144-2152.

Ying S., Durham S.R., Cristopher J.C., Hamid Q. and Kay B. (1995). Phenotype of cells expressing mRNA for TH2-type (Interleukin 4 and Interleukin 5) and TH1-type (Interleukin 2 and Interferone γ) cytokines in Bronchoalveolar lavage and bronchial biopsies from atopic asthmatic and normal control subjects. Am. J. Respir. Cell Mol. Biol. 12:477-487.

Zanders, E.D. (1994). Interleukin-5 alpha chain is downregulated by TGF-β1. Soluble or membrane-anchored ? Eur Cytokine Network 5: 419-422.