Université de Montréal

The Effects of Interleukin-5 (IL-5) on Airway Physiology and Inflammation in Rats

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Thèse présenté à la Faculté des études supérieures
en vue de l'obtention du grade de
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Ce mémoire intitulé :

The Effects of Interleukin-5 (IL-5) on Airway Physiology and Inflammation in Rats

Présenté par :

Samobrata Samuel Nag

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Membre du jury.....**Dr.Andre Caron**

Mémoire accepté le : 7 mai 1999

Sommaire

Les anomalies du système immunitaire au cours de l'asthme sont multiples et paraissent s'amplifier mutuellement sans qu'il soit possible de déterminer une anomalie primitive et de proposer un chéma pathogénique simple les intégrant. Leur interprétation est d'autant plus difficile que les données proviennent souvent d'études ayant porté sur des biopsies obtenues chez des malades à un stade évolué qui ont été traité par des corticoïdes. L'utilisation du modèle animal semble donc approprié pour rensigner sur cette réaction inflammatoire d'origine multifactorielle. Messagers échangés par les cellules, les cytokines semblent être au coeur de toutes les réactions inflammatoires, qu'elles soient médiées par les anticorps ou les cellules. Dans ce contexte, par sa présence en large quantité dans les lavages bronchoalvéolaire des patients asthmatiques, l'interleukine(IL)-5 semble avoir une large contribution dans la physiopathologie de asthme. Dans cette étude nous avons considéré l'effet de l'administration de l'IL-5 exogène sur la physiologie pulmonaire ainsi que l'expression des cytokines (IL-4, IL-5 et IFN-γ) témoin de l'inflammation des voies respiratoires chez le rat. Les rats Brown Norway (BN) qui ne développent pas de réponse aiguë ou semi-retardée après provocation antigénique ont été choisis comme modèle animal dans cette étude. Dans un premier temps la réactivité bronchique à la métacholine suite à l'administration de l'IL-5 par voie intratrachéale a été considéré 14 jours après sensibilisation des animaux à l'ovalbumine (OA) et ceci 30 min, 20h et 72h après l'administration de l'IL-5. La réactivité bronchique à la métacholine augmente 20h après l'administration de l'IL-5, et ceci proportionellement à la dose de l'IL-5. Fait intéressant, le lavage bronchoalvéolaire révèle une présence massive et inattendue de neutrophiles 20h après administration d'une dose d'au moins 3μg d'IL-5. Dans un deuxième temps et 20 heures avant provocation à l'OA, les rats ont reçu une dose d'IL-5 de 3 μg par rapport aux rats contrôles qui ont reçu l'albumine bovin (BSA), l'IL-5 n'a eu

aucun effet sur la réponse aigue ou semi-retardée ni sur l'hyperréactivité bronchique à la métacholine. Cependant, la résistance pulmonaire considérée 20 heures après la provocation antigénique a augmenté chez les rats ayant reçu l'IL-5. L'analyse des cellules totales isolées après digestion pulmonaire et de leur différentielle 8 heures après provocation antigénique ne montra pas de differénce significative, mais le rapport des lymphocytes (Helper/Suppressor) dans le sang était significativement abaissé dans le sang de rats pré-traités avec l'IL-5 ($p<0.05$). L'hybridation *in situ* montra une augmentation significative de l'expression des messagers codant pour l'IL-4 et l'IL-5 à l'intérieur des voies aériennes des rats pré-traités à l'IL-5 et provoqués par l'OA. En conclusion, IL-5 augmente l'hyperréactivité bronchique et la combinasion de l'IL-5 et l'antigène peut causer un augmentation de la résistance pulmonaire et de l'expression des mARN des cytokines Th2.

Summary

There is now clear evidence that IL-5 expression is a prominent feature of airway inflammation in asthma. The aim of this study was to determine whether exogenous IL-5 could affect lung physiology, the late airway response after antigen challenge, airway inflammation and cytokine lung mRNA expression in rats. Brown Norway (BN) rats that do not develop early or late airway responses were sensitized with ovalbumin (OA). Fourteen days later, the effects of intratracheal administration of recombinant human IL-5 were studied. The airway responsiveness to methacholine increased 20 hours after administration of IL-5, at the same time as an increase occurred in neutrophils in bronchoalveolar lavage (BAL). This effect was dose dependent appearing at a dose of at least 3µg of rhIL-5 and only 20 hours but not 30 mins or 72 hours after intratracheal administration. When rats were pretreated intratracheally with 3 µg of IL-5 or control bovine serum albumin (BSA) 20 hours before OA challenge, IL-5 had no effect on the early and late airway responses or on airway responsiveness to methacholine after OA challenge. However, IL-5 increased lung resistance 20 hours after antigen challenge (BSA: 0.09 $cmH_20/ml/s$, IL-5 3µg: 0.18 $cmH_20/ml/s$; $p = 0.01$). Total lung cells and differential counts did not differ significantly 8 hours after antigen challenge, but the blood lymphocyte helper/suppressor ratio decreased in IL-5 pre-treated rats ($p<0.05$) and *in situ* hybridization showed a significant increase in cells within the airway wall expressing IL-4 and IL-5 mRNA in IL-5 treated/challenged rats when compared to controls ($p<0.05$). In conclusion, IL-5 increases cholinergic airway responsiveness and the combination of IL-5 and antigen challenge can lead to an increase in lung resistance and in TH-2 mRNA cytokine expression.

KEY WORDS : IL-5, airway responsiveness, inflammation

Resumé

Asthma is an inflammatory disease of the airways with two characteristics: reversible airway obstruction and increased airway responsiveness (AHR) (1,2). In recent years, it has become clear that infiltration of the airways of patients with asthma by chronic inflammatory cells, particularly lymphocytes and eosinophils, is likely to be of major importance in causing sustained changes in airway responsiveness (143). Activated lymphocytes are present and increased in the airways of patients with asthma (143). These cells seem to be involved in the physiological response that occurs after antigen challenge (145). Indeed, adoptive transfer of antigen-specific $CD4^+$ T lymphocytes induces airway bronchoconstriction and eosinophilia in challenged rats (4). The mechanism by which lymphocytes may be affecting the airway response to antigen is unclear, but may be through one of its mediators, interleukin (IL)-5.

Several lines of evidence suggest that IL-5 may be important in asthma. IL-5 is an important growth factor for eosinophils (124) that is increased in the lungs of allergic and non-allergic asthmatics (12). IL-5 increases in the lungs and sputum of asthmatics 24 hours after antigen challenge (174) and a clear correlation exists between IL-5 expression and the presence of eosinophils in the airways of patients with asthma (149). Intratracheal administration of IL-5 to human asthmatics or to sensitized animals with the characteristics of atopic asthmatics increases the airway response to antigen (151, 157). Although there is compelling evidence for a role of IL-5 in asthma, there is currently no information on whether IL-5 can affect the airway response of normal animals that do not develop the early and late airway response after sensitization and antigen challenge.

In this study we assessed whether administration of IL-5 to RIJ Brown Norway (BN) rats that do not develop early (ER) or late (LR) airway responses after sensitization and antigen

challenge (142) will cause several of the physiological changes that are characteristic of asthma. After determining the optimal concentration of recombinant human (rh) IL-5 that would cause eosinophil differentiation in rats, we assessed the effects of intratracheal administration of IL-5 on airway cholinergic responsiveness, the ER and LR, lung resistance and airway inflammation in these rats.

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Index of Abbreviations

BAL - Bronchoalveolar lavage

IL - Interleukin

IFN - Interferon

TH1 - T helper lymphocyte producing cytokines IL-2, IFN-γ, TNF-β

TH2 - T helper lymphocyte producing cytokines IL-4, IL-5, IL-10, and IL-13

Ig - Immunoglobulin

rh - Recombinant human

AHR - Airway hyperresponsiveness

Mch - Methacholine

BSA - Bovine Serum Albumin

LR - Late phase airway response

ER - Early phase airway response

APC - Antigen-presenting cells

kd - kilodalton

mRNA - messenger ribonucleic acid

VCAM - Vascular cell adhesion molecule-1

MHC - Major histocompatibility complex

VLA - Very late antigen

HLA-DR - Human leukocyte antigen specific for Class II MHC molecules

CD - Cluster of differentiation

PAF - Platelet activating factor

MBP - Major basic protein

ECP - Eosinophil cationic protein

EPO - Eosinophil peroxidase

TGF- Transforming growth factor

GM-CSF - Granulocyte-macrophage Colony stimulating factor

NK - Natural killer

ng- nanograms

g - grams

mg - milligrams

kg - kilograms

ml - millilitre

L - litre

cm - centimetre

CO_2 - Carbon dioxide

CFU-Eo - Colony forming units for eosinophils

OA - Ovalbumin

R_L - Lung resistance

Ptp - Transpulmonary pressure

H_2O - Water

min - Minute

s - Second

PBS - Phosphate buffered saline

TBS - Tris Borate Solution

µg - Micrograms

RPMI - Roswell Park Memorial Institute

SD - Standard deviation

Mch - Methacholine

RIJ - Strain type of Brown Norway rat

SSN - Strain type of Brown Norway rat

1. Introduction

Asthma is a complex syndrome characterized by airway hyperresponsiveness, ɔcalized airway edema with mucus production, and inflammation (1). Symptoms include ɔugh, wheezing and a sensation of chest tightness (2). Allergens are important inducers of rway hyperresponsiveness and related to the development of asthma (3). In patients with ;thma a relationship between genetic predisposition, IgE production, allergen exposure and rway obstruction is well-established (4). Airway obstruction is usually completely reversible hich often occurs with the resolution of the symptoms that are present during an asthma tack. Patients unable to resolve airway obstruction may fall into a state of *status sthmaticus*, which can be fatal. Pathological studies of patients who have died in *status sthmaticus* show a loss of surface lining epithelium, dilation of bronchial blood vessels, ıucosal edema and hypertrophy of both submucosal glands and bronchial smooth muscle. A ɜnse infiltration of inflammatory cells is also present in both the occluding plug and the rway mucosa (5,6). These inflammatory changes are involved in smooth muscle contraction ıd microvascular leakage.

In recent years, there has been a changing view on the pathophysiology of asthma from ;imarily a disease of airway smooth muscle contraction to one of complex interactions ɜtween inflammatory mediators and effector cells. The inflammatory infiltrate is ıulticellular, composed mainly of eosinophils and mast cells, but also contains neutrophils, mphocytes, and mononuclear cells (6,7,8,9). Interestingly, the inflammatory cells are ɔserved to be in an activated state, with degranulated mast cells and eosinophils present .0,11). The release of mediators from inflammatory cells leads to persistence of obstruction

ı the airways.

Analysis of bronchoalveolar lavage (BAL) samples obtained from patients with asthma ıows an increase in activated T lymphocytes, belonging mainly to a Th2 phenotype, based on ıeir cytokine mRNA profile (12). The Th2 cytokines, interleukin (IL)-4 and IL-5, contribute › allergic conditions by directing isotype switching to IgE production and inducing ɔsinophilia in animals and humans (13). As well, studies aimed at decreasing or inhibiting ıe effects of Th2 cytokines, like IL-5, through administration of monoclonal antibodies *in ivo* or the use of animals deficient in these genes, have shown to effectively limit airway yperresponsiveness and airway eosinophil recruitment in allergen-induced animal models of sthma (14, 175).

In my thesis, I will assess whether the administration of recombinant human(rh) IL-5 is irectly related to the airway response seen in asthmatics by performing experiments in rats ıat do not develop the late airway response after antigen challenge. I will assess whether IL-5 an cause by itself all the changes in physiology and inflammation that are encountered in topic asthma. I will thus investigate the effects of rhIL-5 on the late airway response after ensitization and antigen challenge, airway responsiveness to methacholine, and on the ellular influx and mRNA cytokine profile expressed in the lungs after antigen challenge.

.1.1. **Asthma Epidemiology**

Asthma affects 5 to 10% of the population and is most prevalent among children 15,16). The disease is present throughout life, but it tends to improve during adolescence nly to return in older age (17). Most surveys of children in western countries have reported

ıe prevalence of asthma to be higher in boys with some male/female ratios exceeding 2/1 18). Since asthma rarely begins during the first two years of life, and in many patients its nset is delayed until early adulthood or even old age, it would seem that heredity does not irectly determine the inception of bronchial hyperresponsiveness; it is more probable that a redisposition to hyperresponsiveness is inherited, the insult of some environmental factor eing required before it is expressed as asthma (19). Commonly known environmental factors ausing symptoms of asthma include allergens such as cat dander and dust mite and irritants uch as cigarettes and cigarette smoke. Antigens present in the work environment are onsidered environmental factors that may lead to occupational asthma (20).

.1.2. **Asthma Therapy**

Treatment of asthma is mainly focused on two aspects of the disease. The first is to revent or reverse airway inflammation and hyperresponsiveness (21). The second, is to ecrease airway obstruction (22). Therapies have been changing over the years from ronchodilators, to cromoglycates and inhaled corticosteroids. There is little doubt that many sthmatic patients have benefited from corticosteroids, however there are worries concerning :s systemic side effects. It is often forgotten that systemic side effects of consequence to atients come from long term administration and not the short course of treatment which is so ften essential to prevent an asthmatic attack evolving into a severe one which may be life-ıreatening. In acute severe asthma, β-agonists and steroids can be delivered intravenously by ıjection or by a continuous infusion pump (23). In extreme circumstances, intubation and ıechanical ventilation may be necessary despite being hazardous.

Drugs specifically targeting IL-5, a cytokine that causes eosinophil differentiation, roliferation and survival and seems to be involved in eosinophilic bronchitis (a clinical ıanifestation of asthma) (24), are a realistic possibility for the treatment of asthma and other llergic diseases. Experimental work with anti-IL-5 antibodies suggests that anti-IL-5 therapy ıay work in the clinical setting (24).

.1.3. **Intrinsic and Extrinsic Asthma**

Intrinsic (nonatopic) asthma is considered to be a distinct pathological state of asthma, s opposed to extrinsic (atopic) asthma. In intrinsic asthma patients are skin-test negative to ommon aeroallergens and have normal total serum IgE concentrations (25). As well, it ccurs in an older age group of people without any history of allergy to environmental factors 25).

In contrast, extrinsic asthma usually develops in childhood, often occurs seasonally nd remains present throughout life, with periods of remission (26,27). Extrinsic asthma is ighly related to IgE and atopy and patients often have strong immediate hypersensitivity esponses to a variety of allergens (26,28).

.1.4. **Early and Late Airway Responses**

Airway responses in allergic asthma have been categorized into three patterns based on he timing of the response in relation to antigen exposure, namely: 1) early airway response ER); 2) late airway response (LR); 3) bronchial hyperresponsiveness. The ER occurs within ıinutes after inhalation of antigen, lasts up to an hour, and is followed by a prompt return to ıaseline lung resistance (29). The ER is mediated by IgE antibodies, which are present on

ıast cells and basophils. These antibodies cross-link when in contact with allergen leading to egranulation of the cells and increased micrOAscular permeability (30). Bronchoconstriction f the airways during the ER is induced by the release of histamine, leukotrienes, eicosanoids, nd possibly other bronchoconstrictive agents (31).

The LRs have their onset from 4 to 12 hours after antigen exposure and may persist ɔng after antigen exposure has ceased (32). In general, most atopic asthmatics develop both ɛsponses, although occasionally, only an early or a late response is encountered (33). The evelopment of LRs in a given individual appears to depend on several factors. The antigen sed (34), the responsiveness of the airways (35,36), and the production of specific IgE ntibody (37,38,39) all may influence the development of an LR. Airways of individuals xperiencing the LR show increased edema and an infiltration with different inflammatory ells, in particular eosinophils and T-lymphocytes (40,41).

Bronchial hyperresponsiveness is the increased sensitivity and reactivity of the airways ɔ irritants. Hyperresponsiveness can be shown to occur just before the late airway response is ıeasured, within three hours after antigen challenge and can last for several weeks (22). ɛesponsiveness to bronchoconstrictive stimuli appears to be highly variable among ndividuals (42). The increased airway responsiveness is attributed to airway inflammation nd to the mediators that are released by inflammatory cells (43).

.2. The Pathology and Physiology of Atopic Asthma

.2.1. Pathology of Asthma

Characteristic pathologic evidence for inflammation in asthma is routinely observed in utopsy specimens of patients who die of *status asthmaticus*: airway smooth muscle ypertrophy, thickening of the basement membrane, occlusive mucous plug deposition in the erminal bronchioles and the presence of inflammatory cells such as eosinophils, mononuclear ells and neutrophils (8,44,45,46).

.2.2. Airway Inflammation

Inflammation represents the reaction of a vascularized tissue to local injury. For sthmatics, there is evidence of inflammation in the airways as well as inflammatory cells in he BAL and sputum (47,48). Following corticosteroid therapy, there is a reduction in nflammation which led to the hypothesis that inflammatory mediators released by effector ells lead to physiological changes that are seen in asthma (49).

The spectrum of mediators involved in the asthmatic response is diverse. In response o allergen challenge, the human mast cell releases numerous acute phase reactants, including reformed mediators such as histamine (50), adenosine (50), serotonin (50), tryptase (51), and ewly synthesized molecules such as leukotrienes, prostaglandin D_2, thrombaxane, platelet-ctivating-factor and bradykinin (52). Histamine is a major bronchoconstrictor mediator that s released in high levels after antigen provocation (50). Leukotrienes such as leukotriene C_4 ,

eukotriene D_4 and leukotriene E_4 are important in chemotaxis, microvascular permeability nd edema, smooth muscle contraction, mucous hypersecretion and macrophage activation 53,54). Other inflammatory cells can also release mediators. Neutrophils release various xygen radicals such as superoxide anion, hydrogen peroxide, and various proteases (55). Monocytes or macrophages, involved in processing and presenting antigens to other cells, articularly lymphocytes, produce various cytokines that mediate lymphocyte proliferation 56). T-lymphocytes produce different types of cytokines depending partly on the way the ntigen is presented (57). Cytokines comprise a group of mediators responsible for pro-nflammatory effects and can be released from recruited cells later in the course of the esponse. They also influence other inflammatory cells by affecting their state of activation, roliferation and mediator release (58).

.2.3. **Airway Hyperresponsiveness**

Airway hyperresponsiveness is characterized by an increase in sensitivity and reactivity f the airways to factors, such as allergens, and the development of a lower threshold to pasmogens that will hinder airflow (59). Airway hyperresponsiveness occurs before the ymptoms of the LR arise and can remain after the symptoms have subsided (21,22). The type f physiological response is directly related to the degree and method by which various ntigens are presented to the immune system in the airways of atopic asthmatics (43). Exposure to an antigen to which one is sensitized to may only cause an acute, transient ncrease in airway hyperresponsiveness, however continuous exposure to an allergen may ause an individual to reach a chronic state of airway inflammation and hyperresponsiveness

33). Inflammation is not always necessary for airway hyperresponsiveness to occur. Bronchoconstrictive agents such as histamine, cholinergic agents and some β-agonist blockers cause bronchospasm through smooth muscle contraction without airway inflammation (33).

Researchers have long used stimuli, such as histamine and methacholine, to measure the level of airway responsiveness in patients with asthma and in animal models. By instilling or nebulizing incremental doses of these mediators to the airway, they are able to assess and to quantify the threshold of tolerance toward non-specific irritant stimuli (60,61). However a measure of airway hyperresponsiveness is not always considered to be a sign of asthma or even of airway inflammation (62). The amount of airway hyperresponsiveness to cholinergic agonists correlates only with the level of certain asthmatic symptoms such as wheezing and nocturnal cough and can give a limited prognosis of the severity of an asthmatic attack (62). Airway hyperresponsiveness is not restricted to non-specific stimuli. Airway hyperresponsiveness can occur in normal subjects following viral respiratory infection and can be present in atopic non-asthmatic individuals, in patients with chronic obstructive pulmonary disease and in cystic fibrosis (63,64,65).

Airway hyperesponsiveness occurs through neuronal mechanisms and inflammatory mediator release which affects the sensitivity of the airways to stimuli and accordingly the amount of smooth muscle contraction. Consequently, increased airway hyperresponsiveness leads to a decrease in airway diameter (66). The relationship between inflammation and hyperresponsiveness in the airways is especially important during the LR. There is an increased presence of inflammatory cells and mediators in the airways during the LR (67).

.2.4. **Airway Obstruction**

Airflow is impeded during an asthma attack because of thickening of the airway wall s well as contraction of airway smooth muscle (68). These changes lead to a decrease in irway diameter, making it difficult for a patient to breathe through narrower airways (69).

.3. **Inflammatory Cells in Asthma**

The immune system has evolved to mount various lines of defense to foreign particles vithin the body. The first line of defense is the antigen-presenting cell (APC) which hagocytoses the allergen, modifies and presents the peptide to effector cells (lymphocyte) in rder to elicit an appropriate immune response (70). Lymphocytes specific for the antigen nteract with the APC, become activated, and either mediate a humoral (antibody) or cellular delayed-type) response (70). IgM, IgA, and IgG are customary antibodies seen during the umoral response, but in atopic individuals IgE antibodies are also produced leading to the ensitization of the individual to the antigen (71). IgE binds to the surface $Fc\varepsilon R_1$ high affinity eceptors that are present on mast cells and basophils (72). During an immune response, the llergen binds and cross-links the IgE molecules attached to the surface of the cells causing legranulation of the cells and release of preformed and newly synthesized mediators such as istamine, leukotrienes, prostaglandins and others (71).

..3.1. **Eosinophils**

Eosinophils are abundant at sites of immediate hypersensitivity (allergic) reactions and

ontribute to tissue injury and inflammation. Eosinophils are bone marrow-derived ;ranulocytes whose granules contain basic proteins that bind acidic dyes such as eosin. These ;ranules, namely major basic protein (MBP), eosinophil cationic protein (ECP), and osinophil peroxidase (EPO) (73) have cytotoxic properties. In atopic and non-atopic sthmatics, it has been shown that eosinophils are increased within the airway walls (41,74). Aediators contained in eosinophillic granules have been detected in the BAL fluid of active nd non-active asthmatics (9). Many studies have demonstrated that the number of eosinophils n the airways or BAL are correlated with symptom severity, airway responsiveness, and lung unction (75). The growth and differentiation of eosinophils is increased by a helper T cell-lerived cytokine called IL-5, and T cell activation may contribute to eosinophil accumulation t sites of parasitic infestation and allergic reactions. As well, eosinophils have been shown to e a source of various cytokines, such as IL-3, IL-4, IL-5, TGF-β, and GM-CSF (76,77,78,79) Eosinophils and their products are not only important in the pathogenesis of asthma, but may e participating in other diseases like eosinophilic pneumonia and pulmonary fibrosis 80,81,82).

.3.2. **Neutrophils**

Neutrophils respond rapidly to chemotactic stimuli, phagocytose and destroy foreign articles. These cells can be activated by cytokines produced primarily by macrophages and ndothelial cells, and are the major cell population present in the acute inflammatory response 156). There seems to be a limited role for neutrophils in the case of asthmatics. It has been hown that low doses of allergen during the pollen season will cause the recruitment of

eutrophils into the airways of patients (39). Studies suggest that neutrophils have an effect on sthma exacerbations by the production of the neutrophil granule elastase (83). Other studies n primates have suggested that neutrophils have a potential role in the development of the ate airway response and airway hyperresponsiveness (55,77). Neutrophils have been found in he airway submucosa of several patients who died in *status asthmaticus* and in biopsies btained from severe asthmatics (74), but their role in this pathological condition is unclear.

.3.3. **Mast Cells**

Normally mast cells are not found in the circulation. Progenitors present in the bone narrow are believed to migrate to the peripheral tissues as immature cells and undergo lifferentiation *in situ* (84). Mature mast cells possess a high affinity $Fc\varepsilon R_1$ surface receptor or IgE molecules and are important for immediate hypersensitivity reactions. This event is nitiated by the binding of antigen to IgE on the mast cell (85). Administration of the antigen vill cross-link sufficient IgE molecules to trigger mast cell activation. Activation of mast cells esults in regulated secretion of their granules (histamine, heparin), synthesis of lipid nediators (leukotrienes, prostaglandins) and transcription, translation and secretion of ytokines. Studies have shown a significant increase in mast cells in BAL fluids of patients vith mild asthma (48) which seems to correlate with the level of airway hyperresponsiveness 75). Mast cells seem to be important in asthma since their mediators are elevated in the irways of asthmatic subjects when compared to normal subjects even without an allergen hallenge (86,87).

Allergen challenge increases the degree of mast cell degranulation, as indicated by elevated levels of circulating histamine following allergen challenge and a correlation with he development and severity of bronchospasm (88). During the late airway response ncreased levels of mast cells have been identified in the bronchial mucosa (88,89).

.3.4 **Macrophages and Dendritic Cells**

Macrophages are monocytes that have migrated and settled in tissues (90). Dendritic ells come as mature differentiated cells from bone marrow and migrate to different tissues rom the blood (91). Both cell types are antigen-presenting cells, however only macrophages ıave a phagocytic function. Alveolar macrophages and dendritic cells are the first cells that ome into contact with inhaled allergens. The subsequent immune response is heavily lependent on the way the antigen is presented (92). In asthmatic patients, macrophages have een shown to be recruited into the airways (93). Also, macrophages have been detected in ncreased numbers in the BAL fluid of asthmatic patients after antigen challenge during the ate airway response (94). Macrophages can release stored or newly synthesized mediators uch as prostaglandins, leukotrienes, platelet-derived growth factor, PAF, and various ytokines like tumour necrosis factor alpha and IL-1 (95,96). All of these mediators contribute o airway inflammation and hyperresponsiveness and can cause the proliferation of airway mooth muscles. Little is known about dendritic cells other than their ability to present ıntigen.

.3.5. **Lymphocytes**

Lymphocytes are mononuclear cells produced from progenitors in the bone marrow hat can either mature in the bone marrow (B lymphocytes) or the thymus (T lymphocytes) 97). Lymphocytes are important regulators of the immune system by providing a specific and fficient system to destroy and eliminate foreign antigen. Once an antigen is taken by an APC, t is modified and presented to the lymphocyte through MHC antigens and CD4 for helper ells, CD8 for suppressor and cytotoxic cells (97). In addition, T cells express other cell urface proteins which when bound to ligand activate and force the T cell into an effector hase. Without these bonds being formed, lymphocytes may commit to apoptosis programmed cell death), which is characterized by cell shrinkage, DNA fragmentation into ucleosomes and nuclear fragmentation (97).

Lymphocytes are found in the airways of mild and severe asthmatics and are increased ollowing antigen challenge (77). These cells are recruited from the blood into the airways fter antigen challenge (98). Although studies have shown that the number of lymphocytes are imilar in asthmatics and normal individuals (11), it has been shown that there is an increase n activated lymphocytes in BAL of asthmatics (99).

T lymphocytes are divided into two cell populations according to their cell surface narkers, $CD4^+$ or $CD8^+$. $CD8^+$ cells are known as cytotoxic/suppressor T cells. $CD4^+$ cells are nown as helper-inducer cells and in asthma show the ability to regulate 1) IgE production, 2) naturation and activation of inflammatory cells and 3) secretion of various inflammatory nediators (99). When activated, T-helper cells are recruited into the lungs as demonstrated by

he increased expression of their activation markers IL-2R, HLA-DR, and very late antigen VLA)-1 (100,101).

Activation of T cells also leads to increased IL-2 mRNA and receptor expression 102). IL-2 is a major lymphocyte growth factor and inducer of cytokine secretion by T cells 103). IL-2 has been shown to increase airway inflammation and cause airway ıyperresponsiveness in Lewis rats and increased responsiveness to antigen in Brown Norway ats (104,105).

The adoptive transfer of $CD4^+$ T helper cells has been shown to cause a late response o antigen in naive rats (106). The exact mechanism by which $CD4^+$ T cells are able to induce he late airway response is still not clear, but studies show an increase in the variety of nediators produced by $CD4^+$ T cells in the airways of animals challenged with antigen and eceiving adoptive transfer of sensitized lymphocytes (107).

In the mouse, the $CD4^+$ helper T-cell population was found to be divided into two listinct populations based on their cytokine production (108). These subdivisions were dentified by analysis of cytokine production patterns. Th1 cells secrete IL-2, TNF-β and nterferon-γ and are primarily involved in cell-mediated immunity against intracellular ɔathogens, as well as delayed-type hypersensitivity reactions. Th2 cells produce IL-4, IL-5, IL-10, and IL-13 and are responsible for IgE-mediated allergic reactions, as well as phagocyte-ndependent host defense (i.e. against parasites). Both subclasses of T cells produce GM-CSF ınd IL-3. BAL cells from allergic asthmatics contain significantly higher levels of mRNA for IL-3, IL-4, IL-5, and GM-CSF compared with non-atopic, non-asthmatic controls, whereas

evels of IFN-γ are very similar between the two groups (109).

Th2 cells, through their cytokines (IL-4 and IL-13), will cause B cells to switch to plasma cells producing IgE antibodies (110). The increase in activated T helper cells is associated with an increase in low affinity IgE receptor-bearing B cells in the airways of patients with asthma (111). Exposure of these B cells to IL-4 will promote production of IgE antibodies which will induce a type I hypersensitivity reaction in the airways. This reaction will lead to an immediate hypersensitivity response to antigen and accompanying ER and LR 112). Studies show that in sensitized animals serum levels of IgE were found to be elevated and associated with the development of increased airway responsiveness to cholinergic stimuli after antigen challenge (113,114).

..4 **Cytokines**

Cytokines are glycopeptides that are produced transiently by a variety of cells upon activation during an immune response. They have four general functions: 1) mediators of natural immunity 2) regulators of lymphocyte activation 3) regulators of immune-mediated nflammation and 4) stimulators of immature leukocyte growth and differentiation. Cytokines act on different target cells that have receptors for cytokines through an autocrine, paracrine or endocrine mechanism (115).

1.4.1. **Interleukin-4**

IL-4 was initially identified in 1982 as a B cell growth factor (116). It is now known as a regulator of allergic reactions. This 20 kd glycoprotein is made up of 4 alpha helices and 2

ɔeta sheets (117). Its receptor is part of the cytokine receptor family, which shares a common ɔonserved sequence of amino acids WSXWS motif (118).

The principal cellular sources of IL-4 are $CD4^+$ T lymphocytes (119), but other cells ɯch as $CD8^+$ T lymphocytes (119), mast cells (119), monocytes (120) and eosinophils (121) ɯe capable of producing IL-4. Its functions are pleiotrophic, however in an allergic reaction, L-4 stimulates B cells to produce IgE antibodies (110). IL-4 along with accessory subunits on he $CD4^+$ T lymphocyte bind to B cells to promote IgE "switching". IL-4 also inhibits nacrophage activation and blocks most of the macrophage activating effects of IFN-γ, ncluding the release of the mediators IL-1, nitric oxide, and prostaglandins. IL-4 is a growth ɯd differentiation factor for T cells, particularly towards a Th2 subset (122). IL-4 stimulates he expression of adhesion molecules, in particular vascular cell adhesion molecule-1 VCAM-1), on endothelial cells, leading to increased binding of lymphocytes, monocytes, and ɛspecially eosinophils. Selective recruitment of eosinophils and lymphocytes into the airways ɔf patients with asthma has been reported to be through increased VCAM-1 expression (123). L-4 along with IL-3 stimulates mast cell proliferation (119). Studies using *in vivo* and *in vitro* ɯalysis of BAL, bronchial and nasal biopsies, and blood of asthmatics show increased levels ɔf IL-4 mRNA and protein (119,122).

l.4.2. **Interleukin-5**

IL-5 was identified in the mid-70s as a colony stimulating factor for the production of ɛosinophillic colonies (124). It is a 40 kD homodimeric cytokine produced by the Th2 subset

of $CD4^+$ T cells (122), by activated mast cells (125) and by eosinophils (126). Its receptor is part of the WSXWS shared motif family of receptors and the receptor interacts with a 150 kD signal-transducing subunit shared with IL-3 and GM-CSF (127).

The major action of IL-5 is that it acts on bone marrow to induce the proliferation of hematopoietic progenitor cells and differentiation to the eosinophilic lineage (128). The differentiation and growth of these progenitors depends also on the synergistic effect of IL-3 and GM-CSF. IL-5 is a potent activator for eosinophils and basophils (129), enhancing their function and prolonging their survival (130). IL-5 can enhance eosinophil degranulation, antibody dependent cytotoxicity and adhesion to vascular endothelium (124,131). Furthermore, IL-5 and rhIL-5 have been shown to selectively recruit eosinophils to sites of inflammation (132).

Asthmatics show increased levels of IL-5 mRNA and protein in the lungs and the blood (12,125). Administration of IL-5 to guinea pigs by intratracheal injection is associated with increased recovery of eosinophils and neutrophils from BAL (133). In addition, experiments using anti-IL-5 monoclonal antibodies have demonstrated a reduction in airway hyperresponsiveness and eosinophil accumulation in animal models after antigen challenge (134).

1.4.3. **Interferon-γ**

Interferons are classified into two main categories according to their physical properties and sites of production, type I and type II (135). In 1980, interferons were given

greek letter designations, α, β, and γ. IFN-γ is a type I 22 kD glycoprotein that induced an antiviral state and is antiproliferative (136). It is made up of 4 alpha helices and its receptor is made up of two subunits, alpha and beta. The receptor transduces signals via a tyrosine phosphorylation pathway (137). IFN-γ is produced mainly by $CD4^+$ T cells, particularly Th1 cells (138), but can also be produced by $CD8^+$ T cells and natural killer (NK) cells (137). It has a variety of functions, including activation, differentiation, increasing cytotoxic activity of inflammatory cells and enhancing or inhibiting expression of certain molecules on different cells like macrophages, T-lymphocytes, NK cells, B cells, endothelial cells and others (137).

IFN-γ's possible role in atopy and asthma has been to maintain a state of equilibrium within the immune system between IgE production (IL-4 and IL-13) and IgG production (IFN-γ). The levels of IFN-γ mRNA in asthmatic lungs are equal to those in normal lungs, whereas the levels of IL-4 and IL-5 mRNA are increased (138). However, the level of IFN-γ mRNA expression increased when asthmatic patients were treated with steroids and improved (139). In addition, IFN-γ, along with IL-12, a macrophage-derived cytokine, is able to inhibit IL-4 induced synthesis of IgE by acting on transcription of the germ-line ε transcript (140,141). Therefore, IFN-γ is an essential component of the Th1/Th2 balance and immunoglobulin production in allergic reactions.

1.5. **Animal Model used in this study**

In order to study the effects of rhIL-5 after antigen challenge, we used in our experiment a specific strain of sensitized rats. Over the years, the Meakins-Christie

aboratories has used the Brown Norway (BN) rat model as a close reproduction of human ıtopic asthma (142). These rats have shown to replicate several of the characteristics of ıuman atopic asthma such as increased IgE production, ER, LR, airway hyperresponsiveness ıfter antigen challenge, and inflammation and eosinophilia of the airways. However, a study ›y Dr. Turner (142) revealed the existence of two strains of BN rats, namely RIJ and SSN vhich exhibit different reactions to antigen challenge depending on their strain. She was able o identify the RIJ strain of BN rat as an evolved non-allergic model of asthma; incapable of nounting an early or late response to allergens, and show a decreased airway ıyperresponsiveness to methacholine compared to SSN BN rats (142). We decided to use the RIJ strain of BN rat to study if the administration of rhIL-5 prior to antigen challenge was able o reproduce the major characteristics of human atopic asthma in rats that do not usually levelop these characteristics. Results from this study would determine the role that IL-5 has in he airway response following antigen challenge.

Materials and Methods

2.1 Eosinophil colony proliferation from peripheral blood mononuclear cell progenitors PBMNC)

These experiments (n = 10) were performed to assess whether rhIL-5 induced eosinophil proliferation from progenitors in rats as previously described in humans (143). Peripheral blood mononuclear cells were isolated from the blood of ten BN rats by centrifugation over Ficoll Hypaque, resuspended in culture medium and plated at a concentration of 1 x 10^6 cells per ml in 35 x 10 mm tissue culture dishes (Falcon) in supplemented Iscove's modified Dulbecco's medium containing 20% heat inactivated fetal bovine serum, 1% penicillin, 1% streptomycin, 5 x 10^{-5} mol/L 2-mercaptoethanol, 0.9% methylcellulose in medium alone or with rhIL-5 (0.25, 1, 2 or 5 ng/ml). After 14 days of culture at 37°C in a humidified atmosphere containing 5% CO_2 in air, colonies (defined as any aggregation of more than 40 cells) were counted by inverted microscopy and eosinophil-type colonies (CFU-Eo) were counted as a function of their morphologic appearance. CFU-Eo appeared as tight, compact aggregations of round, refractile cells and showed varying combinations of eosinophilic, basophilic, and mixed eosinophilic-basophilic cells on May-Grunwald-Giemsa staining.

2.2 Animals and sensitization

Seventy-four highly inbred male BN RIJ rats, 7-8 weeks old and weighing 200 to 240 g were obtained from Harlan Sprague-Dawley Inc. (Walkerville, MD). Rats were maintained in conventional animal facilities at the Meakins-Christie Labs of McGill University. All procedures in

his study were approved by the University Animal Care Committee.

Active sensitization was performed in all rats by subcutaneous injection of 1ml of saline containing 1 mg of Ovalbumin (OA) and 200 mg of aluminum hydroxide (Sigma Chemicals, St.Louis, MI). At the same time 1 ml of *Bordetella pertussis* vaccine containing 5 x 10^9 heat-killed organisms was given intraperitoneally as adjuvant.

2.3. **Measurement of lung functions**

General anesthesia was induced with either pentothal (50 mg/kg) or urethane (1.1 g/kg) intraperitoneally. Blind endotracheal intubation was then performed using a 6-cm length of PE-240 polyethylene catheter. A heating pad was used to maintain body temperature constant, and rectal temperature was monitored continuously with an electronic thermometer (Telethermometer; Yellow Springs Instrument Co., Yellow Springs, OH). Lung resistance (R_L) was measured during spontaneous tidal breathing with the animals in the lateral decubitus position. Flow was measured by placing the tip of the tracheal tube inside a small Plexiglas® box (265ml in volume). A Fleisch no. 0 pneumotachograph coupled to a differential transducer (MP-45 $\pm$ 2 cm H_2O; Validyne Corp, Northridge, CA) was attached to the other end of the box to measure airflow, and volume was obtained by numerical integration of the flow signal. Changes in esophageal pressure were measured using a saline-filled catheter and a differential pressure transducer (Sanborn 267 BC; Hewlett-Packard, Waltham, MA). The other port of the transducer was connected to the box. The esophageal catheter consisted of polyethylene tubing (PE200) 20-cm long attached to a shorter length (6cm) of tubing (PE 100). Transpulmonary pressure (Ptp) was computed as the difference

›etween esophageal and box pressure. The airway response was evaluated from R_L, which was letermined by fitting the equation of motion of the lung by multiple linear regression using ›ommercial software (RHT-Infodat Inc, Montreal, Quebec, Canada). Endotracheal tube resistance vas 0.11 cm H_2O/ml/s at a flow of 25 ml/s. Tube resistance was subtracted from all values of R_L.

!.3.1. **Airway responsiveness to methacholine (Mch)**

Rats were anesthetized with pentothal (50 mg/kg) , intubated and baseline R_L was neasured. The rats were given an aerosol of phosphate-buffered saline (PBS) followed by ›rogressively doubling concentrations of Mch (from 0.0625 to 32 mg/ml in sterile saline) and R_L vas measured after each aerosol until it increased to at least 200% of the PBS baseline value. Aerosols were generated from 3ml of solution using a Hudson nebulizer with an airflow of 10L/min; each administration lasted for 30 seconds.

2.3.2. **Measurement of airway responses to ovalbumin (OA)**

Fourteen days after sensitization, rats were anesthetized with pentothal (50 mg/kg), endotracheally intubated and received either IL-5 (3µg) or the same weight of BSA intratracheally ›rior to being awaken. The following day, rats were anesthetized with urethane (1.1 g/kg), ntubated and baseline R_L was measured. The rats were then challenged with aerosolized OA (50 ng/ml in sterile water) with a Hudson nebulizer (Model 1400; Hudson, Temecula, CA) at an airflow of 10L/min for 5 minutes. R_L was measured before and at 5, 10, and 15 min after the OA challenge, and at 15 min intervals for a total period of 8h.

2.4. **Bronchoalveolar lavage (BAL)**

These experiments were performed 14 days after sensitization. Thirty-two rats were anesthetized with pentothal (50 mg/kg), endotracheally intubated and were awakened after intra-tracheal injection of rhIL-5 or different concentrations (1 to 10μg) of BSA. Twenty hours later the animals were again anesthetized with pentothal, intubated and BAL was performed after methacholine challenge. The lungs were lavaged through the tracheal tube by five instillations and immediate retrieval of 5ml of saline at room temperature. The cells were separated from the supernatant by centrifugation, washed and the total cell count was determined with a hemacytometer. The differential cell count was assessed on a cytospin slide that was prepared with a Cytospin model III (Shandon, Pittsburgh, PA) and stained with Wright-Giemsa. At least two hundred cells were counted under light microscopy (X200).

2.4.1. **Staining of BAL cells for major basic protein (MBP)**

Cells obtained from BAL were fixed in acetone-methanol and incubated with 60μl of primary monoclonal MBP IgG antibody (diluted 1:30 in Tris Borate Solution (TBS), (R&D Systems, Minneapolis, MN)) in a humid chamber overnight at 4°C. The slides were washed and then stained as previously described (144) using APAAP and alkaline phosphatase followed by counterstaining with Harris Haematoxylin. Slides were then dried in the oven at 37 °C overnight and the next day, a coverslip was placed on top of the crystal mount film and the cells were viewed with a light microscope (X200).

2.4.2. **Lung mincing and digestion**

These experiments were performed 8 hours after OA challenge as previously described 143) in 16 rats either pretreated with 3 µg of IL-5 or 3 µg of BSA. The chest wall was opened, ınd the animal was exsanguinated by puncture of the left ventricles and section of the abdominal ıorta. Blood was kept in a heparinized tube for analysis of lymphocyte subsets by flow cytometry. The pulmonary vasculature was washed by slowly injecting balanced salt solution (10ml) into the ight ventricle until the lungs were white. The lungs were dissected from the chest and mediastinal tructures, weighed, and separated into the large airways (trachea and large bronchi until ıpproximately the fifth generation) and small airways and parenchyma (S/P). Tissue digestion and ell retrieval through a #60 sieve (Sigma) was performed and slides were prepared by centrifuging 500,000 cells in enriched RPMI 1640 for 5 min at 400 rpm in a cytocentrifuge. The cellular lifferential was assessed on a Wright-Giemsa stained slide by counting 200 cells under oil-mmersion microscopy (X200)

2.4.2.1. **Isolation and identification of blood lymphocyte subsets**

Peripheral blood mononuclear cells were isolated from fresh heparinized peripheral blood ›y standard Ficoll-Hypaque methods and prepared for flow cytometry as previously described 145). Briefly, the isolated cells were washed with Hanks balanced salt solution (HBSS) and ıtained directly by incubating with the monoclonal antibodies W3/25 (equivalent of the CD4 ıelper lymphocytes), OX8 (CD8 suppressor/cytotoxic lymphocytes) for 30 min. Cells were studied mmediately or fixed in 1% paraformaldehyde/0.85% saline. The fixed cells were stored at 4 °C in

he dark until analysis. Flow cytometry was performed with an argon laser, 488nm FACScan nalyzer (Becton Dickinson FACS Division, Sunnyvale, CA) focusing on the lymphocyte cluster. Controls consisted of cells obtained from mouse ascites fluid and stained with IgG_1 antibodies lirected against CD45 antigens.

.4.3. **Detection of cytokine mRNA expression**

These experiments were performed 20 hours after OA challenge as previously described 143) in 16 rats either pretreated with 3 µg IL-5 or BSA. All animals were killed by xsanguination, and the pulmonary vasculature was washed by injecting balanced salt solution into he right ventricle until the lungs were white (± 10ml). The lungs were dissected from the chest, ixed in 4% paraformaldehyde and then transferred an hour later into a 15% sucrose in PBS olution at 4°C.

Airways from the lung were cut transversely into 1cm pieces, and blocked with liquid itrogen. Cryostat blocks were cut at a depth of 10µm/ section, were placed on poly-L-lysine oated slides and stored at -80°C.

In situ hybridization was performed as previously described (146). Briefly, the cDNA for L-4, IL-5 and IFN-γ was subcloned into the Bluescript RNA vector (pBluescript SK-; Stratagene, a Jolla, CA) and sense and antisense probes were generated by T7 and SP6 RNA polymerases. abeling of RNA probes with digoxigenin-11-UTP was performed according to the manufacturer's ecommendations (Boehringer Mannheim, Mannheim, Germany). The labeled probes were ligested by alkaline hydrolysis to an average length of 100 to 200 bases before precipitation and

ıybridization was performed at 42°C for 12h. The slides were washed in decreasing concentrations ıf SSC (4x SSC to 0.1x SSC) and RNase A (20µg/ml) to remove unhybridized probe. As a ıegative control, preparations were hybridized with DIG-UTP-labeled sense probes under the same :onditions. Specimens were then stained by the colour reaction of a buffer complex (45 µl NBT 4-nitroblue tetrazolium chloride, 75 mg/ml in 70% dimethylformamide, Sigma), 35µl X-)hosphate (BCIP, 5-bromo-4-chloro-3-indoyl-phosphate, 50mg/ml in dimethyl formamide, Sigma)) per 10 ml of buffer (100 mM Tris-HCl, 100 mM NaCl, 5 mM $MgCl_2$, pH 9.5) until the lesired intensity was reached. The alkaline phosphatase reaction was stopped by a change to a)uffer containing 10mM Tris, 1mM EDTA, pH 8.0. Visualization under a Zeiss Axiophot luorescence microscope (Carl Zeiss (Oberkochen), Ltd., Welwyn Garden City, U.K.). Positive :ells were counted in a random coded order at x200 magnification. In the airway submucosa,)ositive cells were counted along the entire length of the epithelial basement membrane in a ninimum of 6 sections. The percentage of cytokine-positive cells was calculated, and results are 'eported as the mean ± SD.

2.5. **Statistical analysis**

The concentration of Mch required to double R_L ($EC_{200}R_L$) was obtained by linear nterpolation between the two concentrations bounding the point at which R_L reached 200% of the :ontrol value. Comparisons of airway responsiveness to Mch between groups were performed with og transformed data. To compare the prevalence of ER among treatment groups, we defined a ;ignificant ER as an increase in R_L to at least 150% of the baseline value within 1 hour after OA

challenge. The late response was calculated as the area under the R_L vs time curve from 180-480 min. post challenge. The differences between the tested and control group means were analyzed using the Student's t test or the Mann-Whitney U-test, as appropriate, to compare magnitude, time to peak, ER and LR, inflammatory cells and cytokines. Results are presented as mean $\pm$ SEM, except for values of $EC_{200}R_L$, which are reported as geometric means. To determine the statistical significance of the airway responsiveness to MCh challenge, we employed Fisher's exact test. Significance was accepted when the probability (p) value was ≤ 0.05.

2.6. **Chemicals**

Ovalbumin, BSA, APAAP, urethane, methacholine, paraformaldehyde, collagenase, and Wright-Giemsa stain were purchased from Sigma Chemicals (St.Louis, MI); *Bordetella pertussis* vaccine was purchased from the Armand Frappier Institute (Montreal, Quebec, Canada); Fetal calf serum, penicillin, streptomycin, L-glutamine, nonessential amino acids, RPMI 1640 medium, Iscove's modified Dulbecco's medium, HBSS and tryptan blue were obtained from GIBCO Laboratories (Grand Island, NY). Ficoll-Hypaque was obtained from Pharmacia (Montreal, Quebec, Canada). rhIL-5 was graciously donated by Merck Frosst (Montreal, Quebec, Canada). Penthothal was obtained from BDH Pharmaceuticals (Montreal, Quebec, Canada). Harris Haematoxylin was purchased from Zymed Chemicals (California, USA), rompun was bought from Bayer (Etobico, Ontario) and somnotol was bought from MTC Pharmaceuticals (Cambridge, Ontario). W3/25 and OX8 was obtained from DAKO Cedarlane (Carpinteria, CA).

tesults

.1 Recombinant human IL-5 administration: experimental results

.1.1 Effect of rhIL-5 on eosinophil colony formation from progenitors

Rat PBMNC's (n = 10) were incubated in 0, 0.25, 1, 2, and 5 ng/ml of rhIL-5 in enriched tPMI medium for 14 days. Concentrations of IL-5 above 1 ng/ml caused a significant increase in osinophil/basophil colonies when compared to controls ($p<0.05$) and the number of osinophil/basophil colonies increased with the concentration of rhIL-5 (figure 1).

.1.2 Effect of intra-tracheal administration of rhIL-5 on airway responsiveness to nethacholine

Bovine serum albumin, 1, 3 or 10µg of rhIL-5 was administered intratracheally and airway esponsiveness to Mch was measured 20 hours later. The amount of Mch that caused a doubling in R_L (lung resistance) decreased significantly as doses of IL-5 increased from 1µg to 10µg (figure 2). The airway response to Mch was significantly increased in the rats that received 3 µg of IL-5 when compared to the group that received BSA ($p< 0.05$). In addition, the airway responsiveness to Mch hat occurred 20 hours after pretreatment was significantly increased in the group that received 10 ıg of rhIL-5 when compared to the BSA and 1µg group (1.3 ± 0.4 mg/ml vs 3.4 ± 2.3 mg/ml vs 6.5 ± 0.9 mg/ml for 10µg vs 3µg and BSA , respectively; $p<0.01$). Airway responsiveness to Mch 30 nin. and 72 hours after administration of 10µg of IL-5 administration was not significantly ıffected. (figure 3)

.1.3. **Effect of dose of intra-tracheal rhIL-5 on inflammatory cells from bronchoalveolar ıvage**

Intra-tracheal administration of rhIL-5 increased the cellular return from BAL 20 hours fter administration (n=32). The total cellular return was for 0µg: 6.65 ± 2.08 x 10^6 cells, for µg: 9.75 ± 1.84 x 10^6 cells and for 10µg: 13.1 ± 3.07 x 10^6 cells ($p<0.05$). The number of eutrophils increased significantly in the rats that received 10µg of IL-5 when compared to the roup that received no IL-5 (10 µg IL-5: 8.01 ± 2.21 x 10^6 cells vs. no IL-5: 2.78 ± 0.73 x 10^6 ells; $p<0.05$). No difference was found in the total number of macrophages, lymphocytes, and asophils that were present in the BAL of each group (Table 1). There was also no difference ı total eosinophils between the groups when evaluated by Wright Giemsa stain or with an nti-MBP stain.

.2 **Recombinant human IL-5 administration & Ovalbumin challenge; Experimental results**

.2.1. **Effect of rhIL-5 on the BN rat physiological airway response after Ovalbumin hallenge**

Pretreatment with 3µg of rhIL-5 20 hours prior to antigen challenge did not affect the ER Figure 4A) or LR (Figure 4B) but significantly increased R_L 20 hours after challenge with OA (IL- : 0.178 ± 0.046 cm H_2O/ml/s vs. Control : 0.094 ± 0.057 cm H_2O/ml/s, $p = 0.01$) (fig. 5). Airway esponse to Mch was unaffected 20 hours after challenge with OA (IL-5: 17.9 ± 3.47 $EC_{200}R_L$ vs.

Control : 11.80 ± 2.93 $EC_{200}R_L$, $p < 0.05$) (Fig.6). When we calculated the amount of Mch necessary to cause an increase in R_L of 0.1 cm H_2O/ml/s, there was also no difference between groups. (IL-5 : 17.7 ± 15.75 vs. Control : 10.64 ± 6.20, $p = 0.33$).

.2.2. **Effect of rhIL-5 on blood lymphocyte subsets 8 hours after Ovalbumin challenge**

We found that pretreatment with rhIL-5 caused a significant change in lymphocyte subsets in the blood of BN rats 8 hours after OA challenge. We found a higher number of $CD8^+$ cells in IL-5 treated and challenged rat blood (mean control: 20.6 ± 5.8 vs. challenged: 30.6 ± 3.7, $p < 0.05$), while the mean number of $CD4^+$ cells was lower (control mean: 43.0 ± 6.4 vs. challenged: 32.8 ± 3.2, $p < 0.05$). The CD4/CD8 ratio in the blood of IL-5 treated and challenged rats significantly decreased when compared to BSA pretreated rats (IL-5: 1.18 ± 0.47 vs BSA:2.92 ± 0.57 , $p < 0.05$) (Figure 7).

.2.3. **Effect of IL-5 on lung cytokine expression after Ovalbumin challenge**

We found an increase in Th2 cytokines expressed in the airways of IL-5-treated rats compared to Th1 cytokines (Table 2). Digoxigenin-UTP RNA probes were used on 8μm thick airway sections from animals treated with rhIL-5 and controls. Slides were stained with the NBT-X-phosphate stain and counted as a representation of 8 slides per group.

egends

igure 1:

ffect of rhIL-5 on eosinophil/basophil type colony production in rat PBMNC's (n=10). Cells ere cultured in medium alone or in different concentrations of rhIL-5 for 14 days at 37°C and olonies were counted by inverted microscopy after May-Grunwald-Giemsa staining. * $p<0.05$ etween IL-5 groups (1, 2, and 5ng/ml) and control.

igure 2:

ffect of dose of intratracheal IL-5 on the airway response to methacholine 20 hours after ntigen challenge (n=12). * $p < 0.05$ between IL-5 treated and BSA-treated (control). ** $p < 0.01$ etween IL-5 treated and BSA-treated (control).

igure 3:

ffect of 10µg of IL-5 on airway responsiveness to Mch 30 min. and 72 hours after dministration. Rats (n=20) received either 10 µg of rhIL-5 or BSA (control) intratracheally nd were both given doubling doses of nebulized methacholine until baseline R_L doubled. $PC_{200}R_L$ was calculated as the amount of methacholine necessary to double R_L.

igure 4:

ffect of pre-treatment with rhIL-5 on the ER (A) and LR (B) after antigen challenge. Rats n=16) were sensitized to OA and received either 3 µg of rhIL-5 or BSA intratracheally. 20 ours later, rats were challenged with OA and R_L was measured for 8 hours. The ER was

alculated using the highest R_L value for 1 hour after antigen challenge. The LR was calculated or R_L values obtained from 4 to 8 hours after antigen challenge.

igure 5:

ffect of rhIL-5 on lung resistance 20 hours after antigen challenge. * $p<0.05$ between IL-5 reated/challenged and control groups. Rats (n=16) were given 3 µg of rhIL-5 or BSA and hallenged with OA 20 hours later. R_L was measured 20 hours after antigen challenge in both roups of rats.

igure 6:

ffect of rhIL-5 on the airway response to methacholine 20 hours after antigen challenge. Rats n=8) were given 3 µg of rhIL-5 or BSA and challenged with OA 20 hours later. Both groups of rats were given exponentially increasing doses of nebulized methacholine until baseline R_L loubled. $EC_{200}R_L$ was calculated as the amount of methacholine necessary to double R_L.

igure 7:

ffect of rhIL-5 on lymphocyte subsets in the blood 8 hours after antigen challenge. Rats n=16) were sensitized with OA and received either 3 µg of rhIL-5 or BSA intratracheally. 20 ours later, rats were challenged with OA and R_L was measured for 8 hours. Peripheral blood vas recovered and analysed by flow cytometry for the percentages of $CD4^+$ (helper) and $CD8^+$ suppressor) lymphocytes. The CD4/CD8 ratio is presented for 10 rats in each group. * $p <$).05 between IL-5 treated/challenged and control groups.

Figure 1

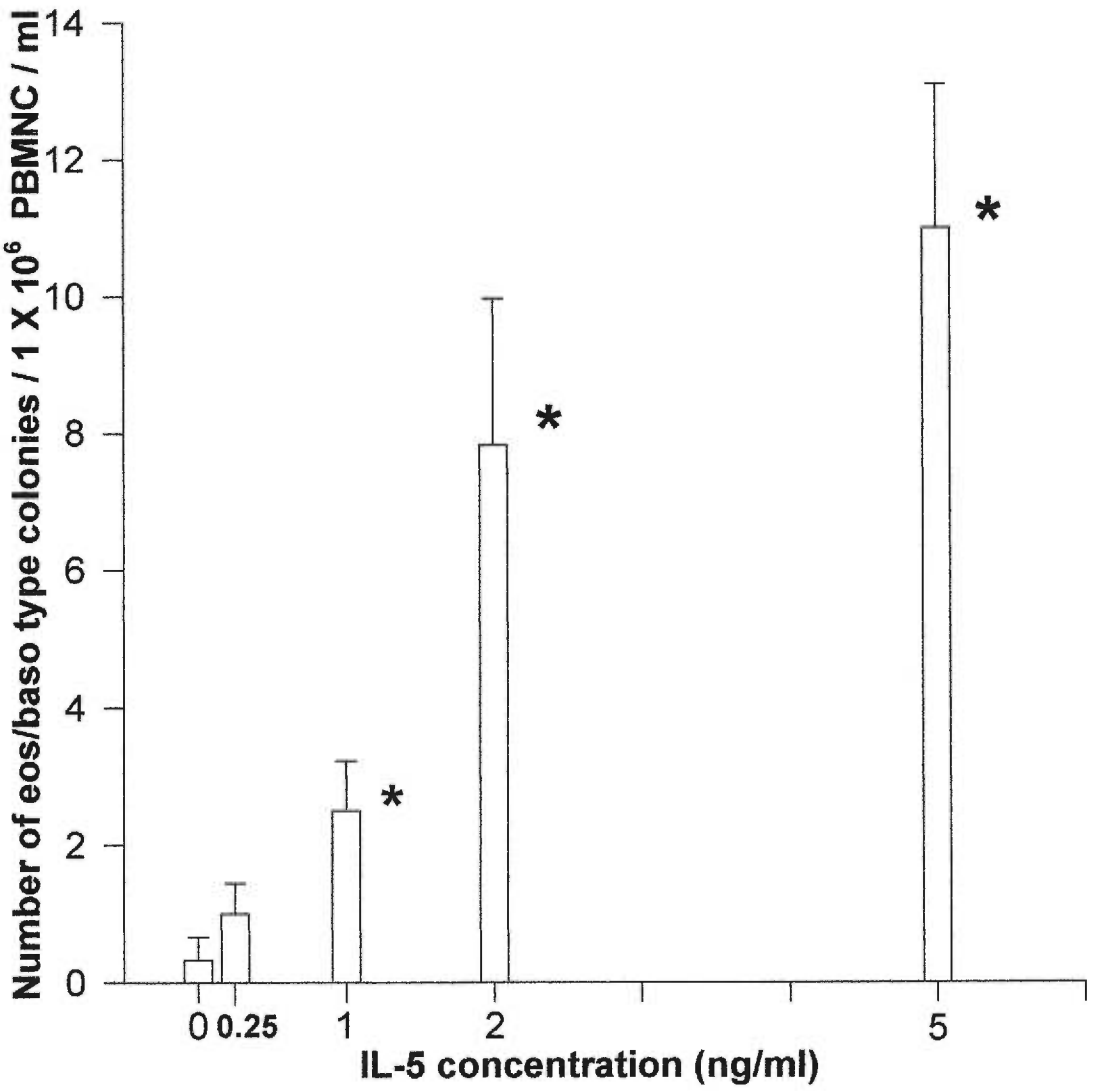
Number of eos/baso type colonies / 1 X 10^6 PBMNC / ml
14
12
10
8
6
4
2
0
0
0.25
1
2
5
IL-5 concentration (ng/ml)
*
*
*

Figure 2

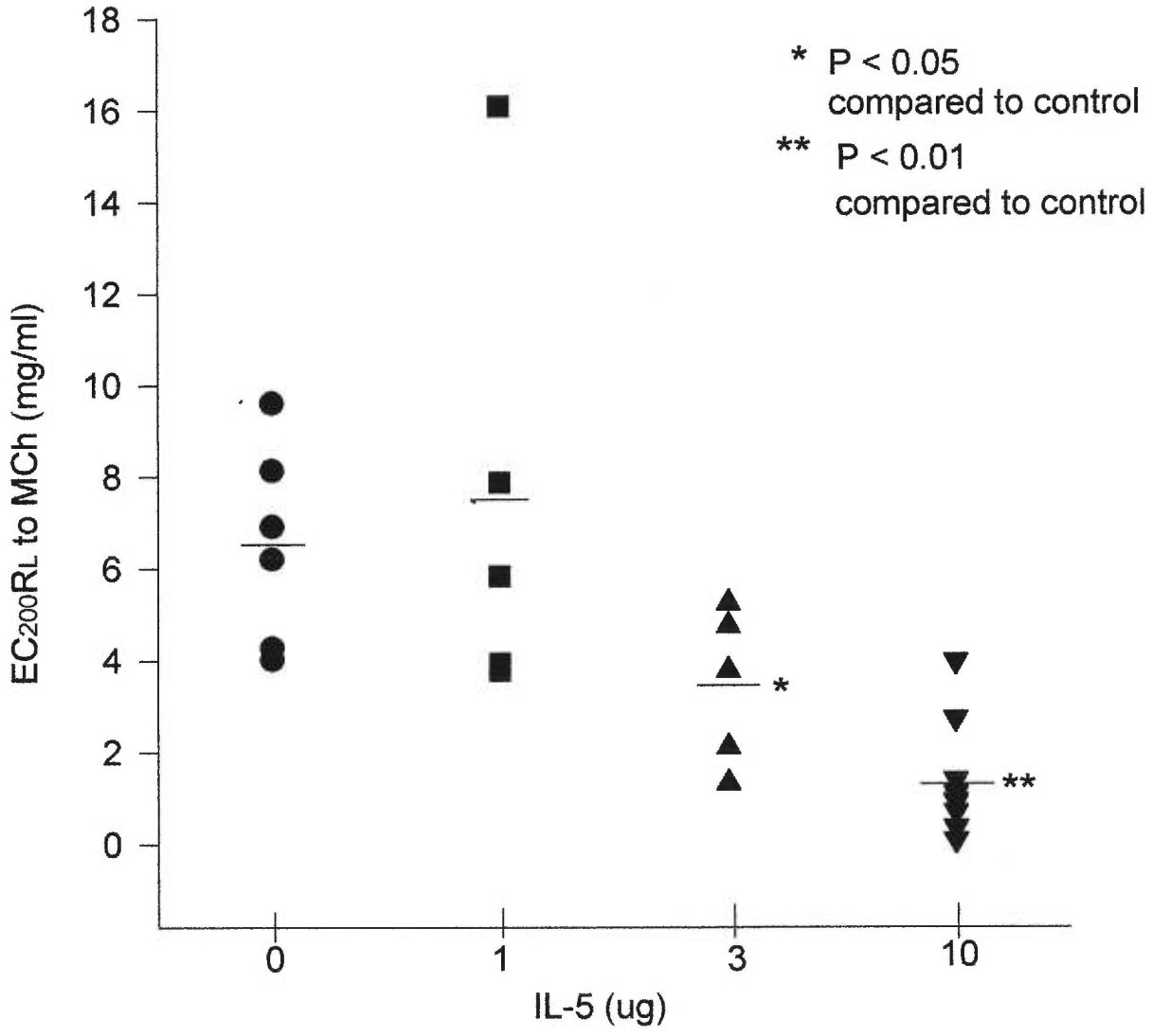
18
16
14
12
10
8
6
4
2
0
EC200RL to MCh (mg/ml)
0
1
3
10
IL-5 (ug)
* P < 0.05
compared to control
** P < 0.01
compared to control
*
**

Figure 3

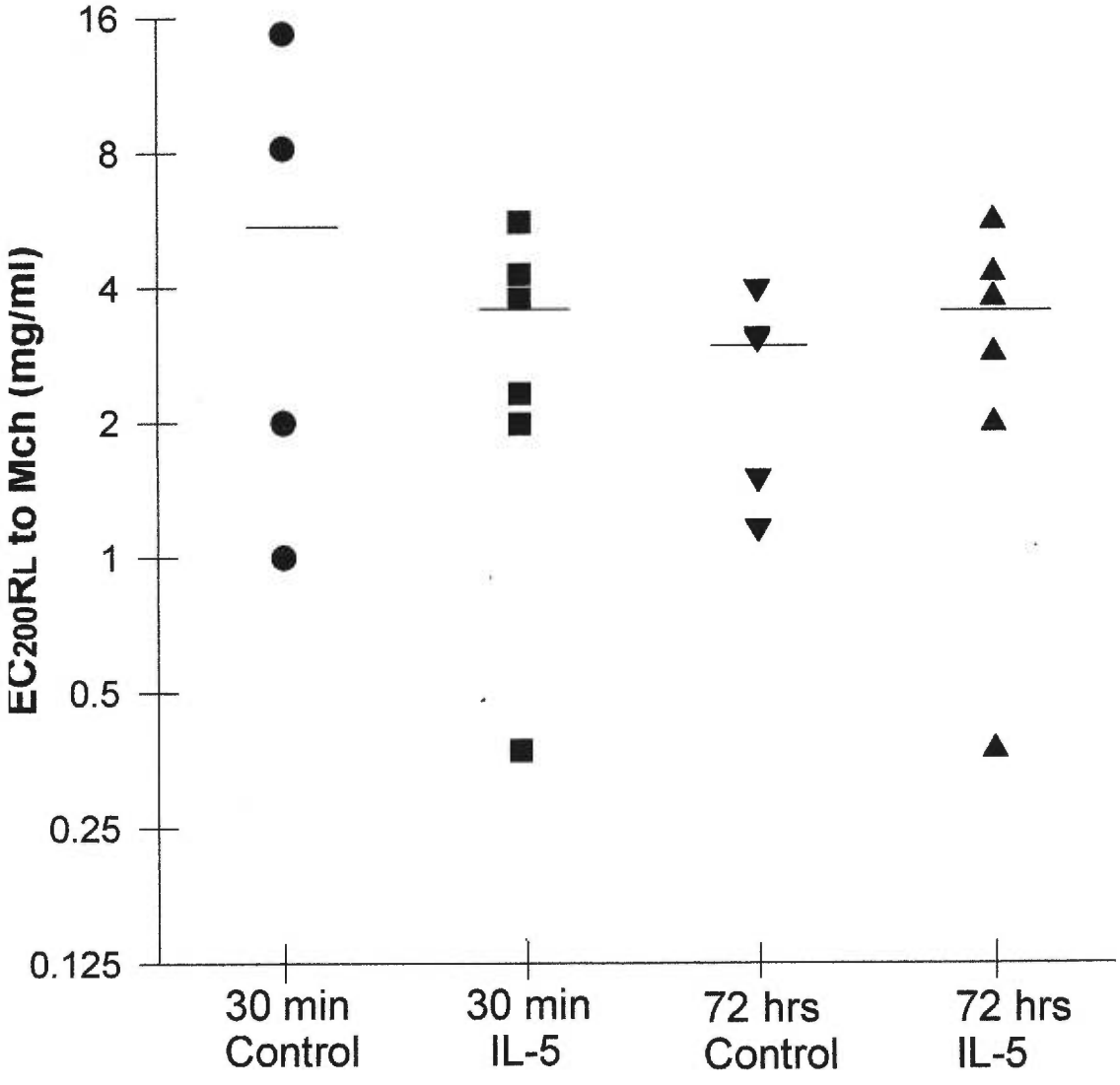
16
8
4
2
1
0.5
0.25
0.125
EC200RL to Mch (mg/ml)
30 min Control
30 min IL-5
72 hrs Control
72 hrs IL-5

Figure 4A

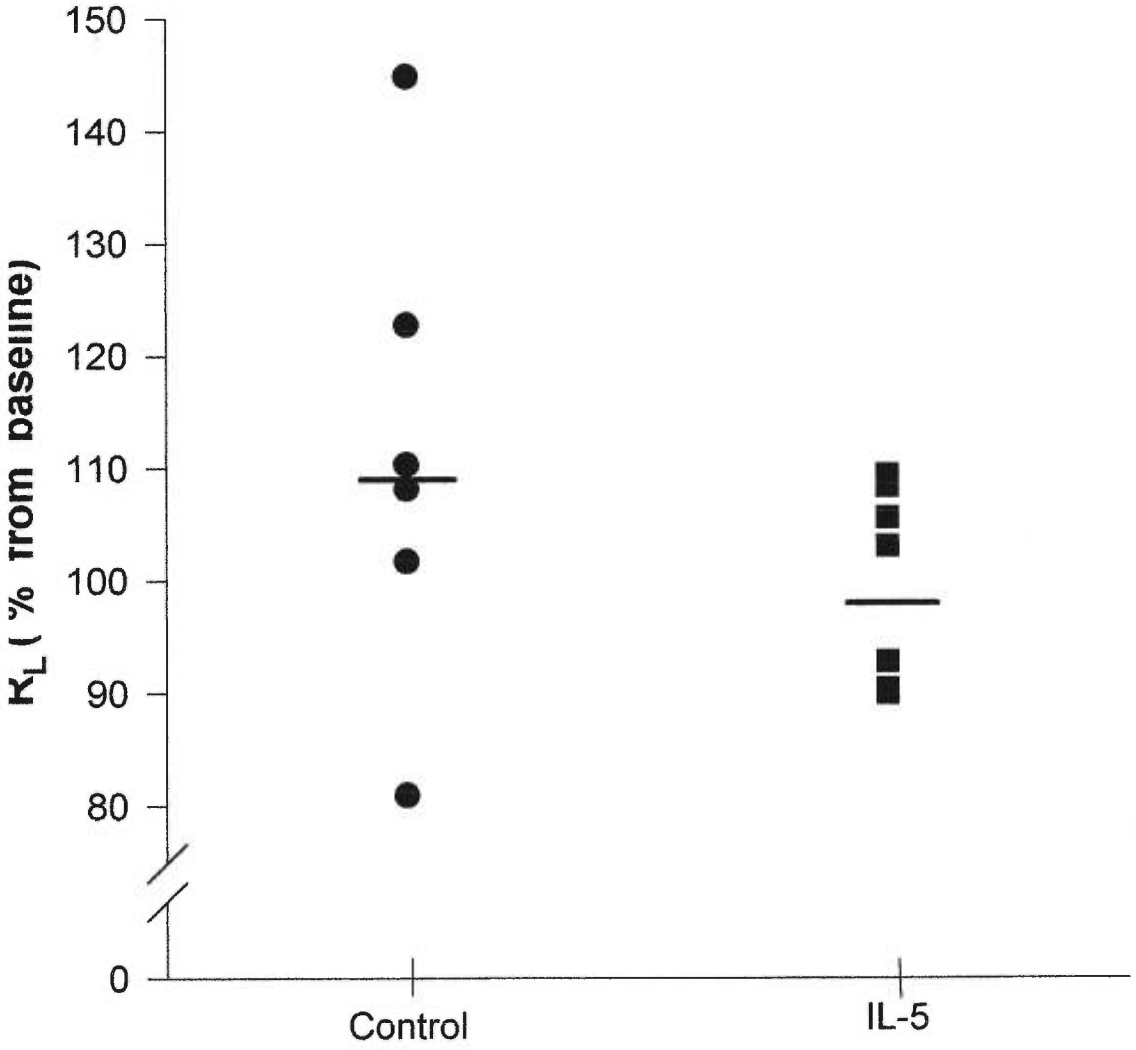

Figure 4B

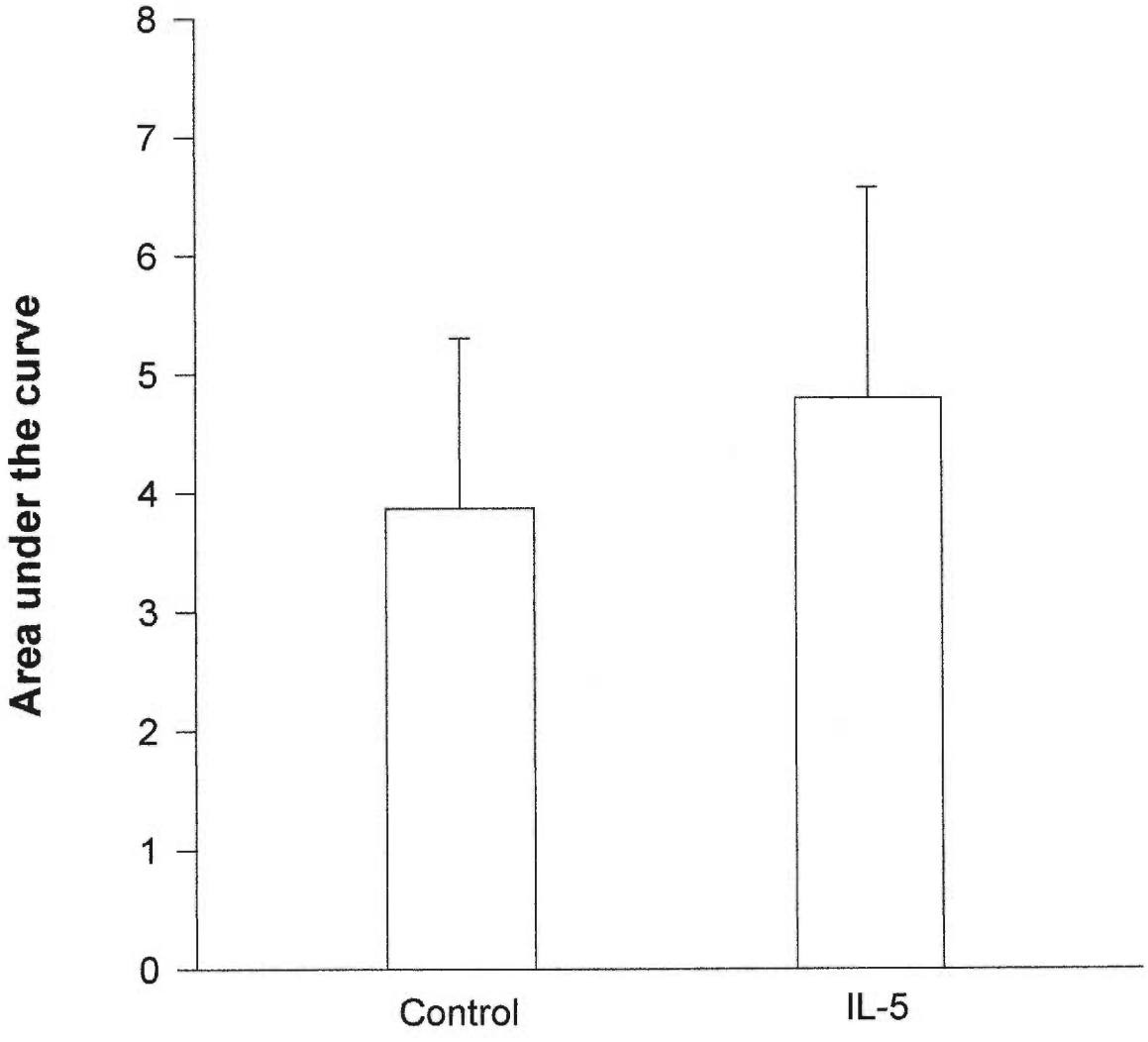

Figure 5

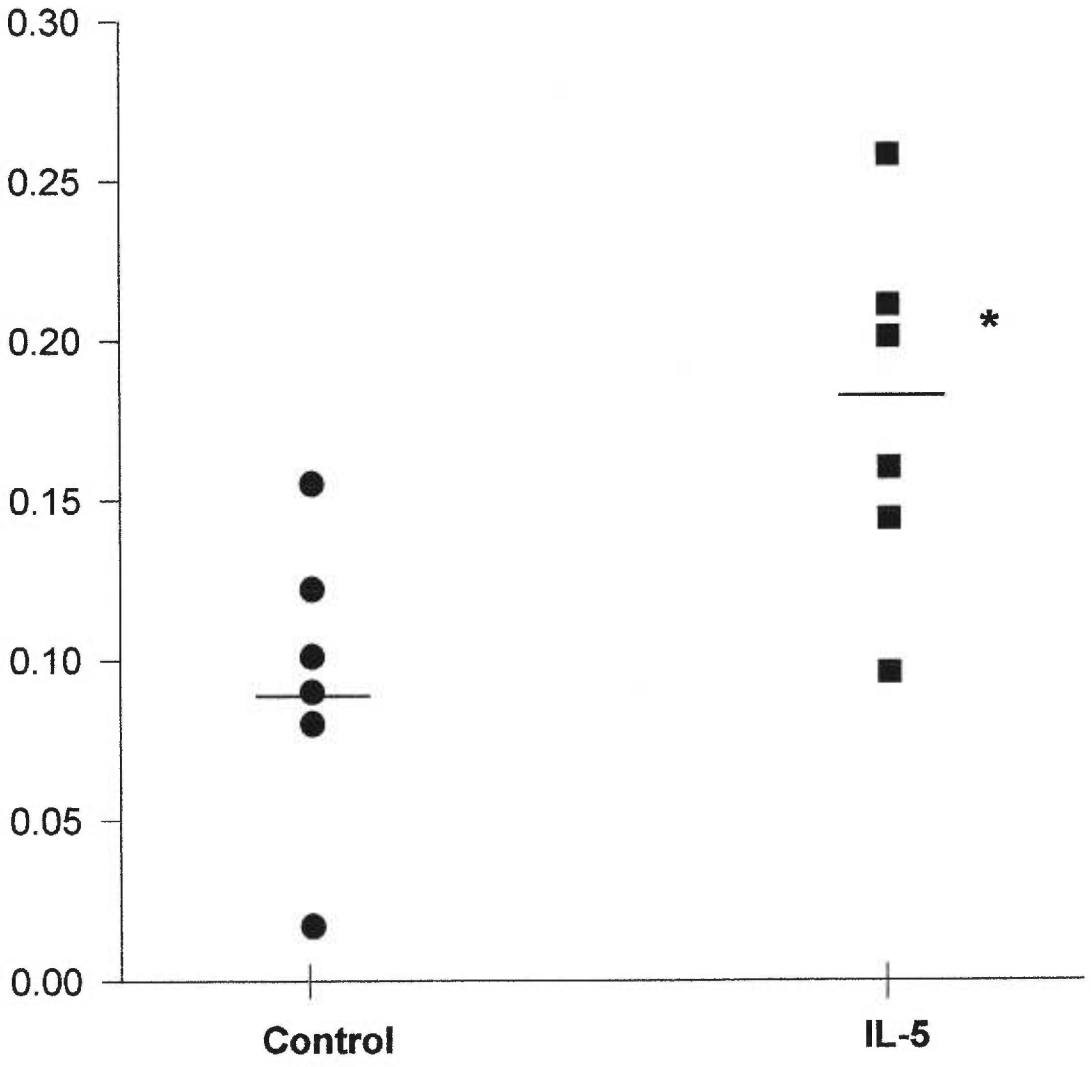

Figure 6

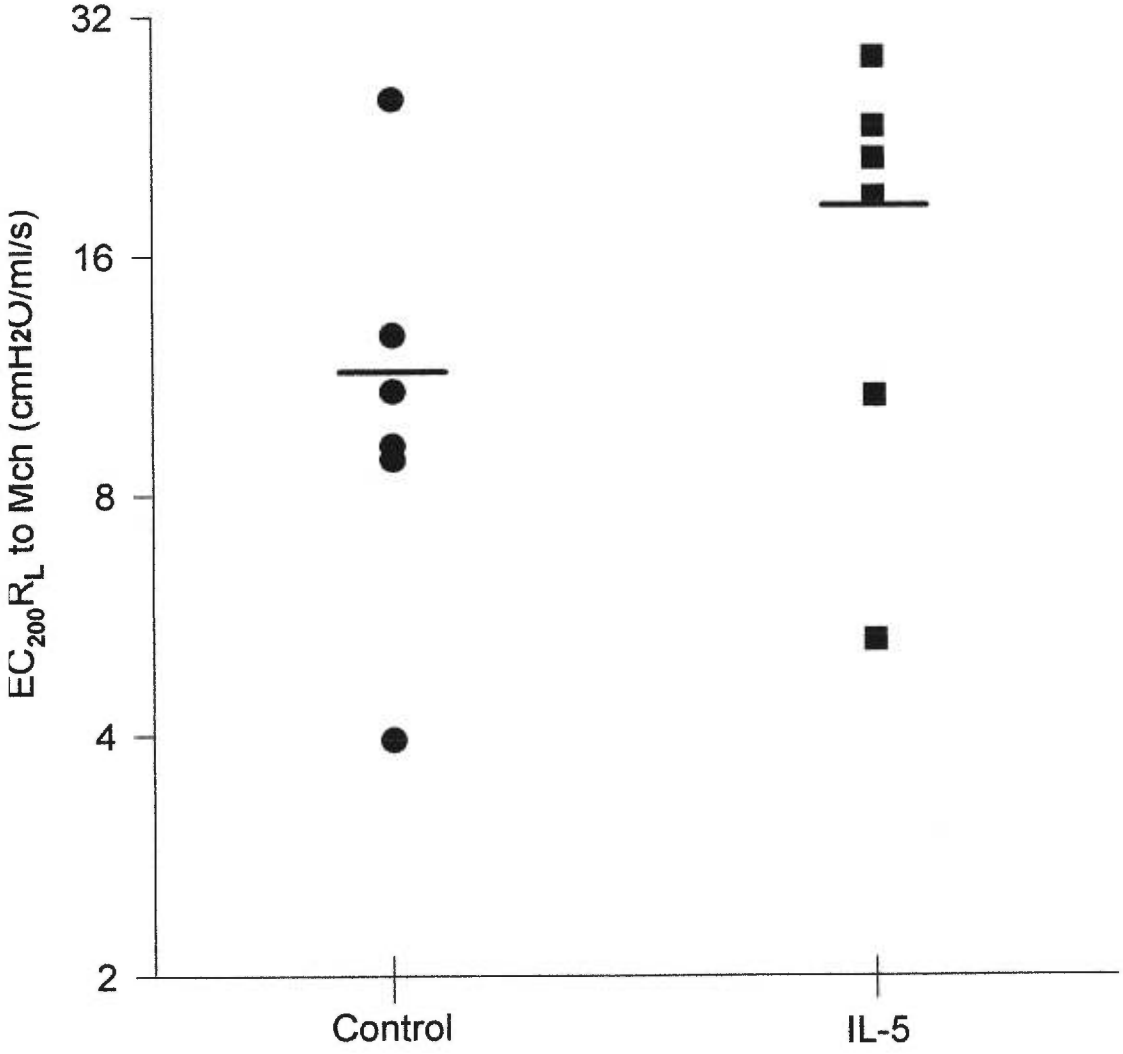

Figure 7

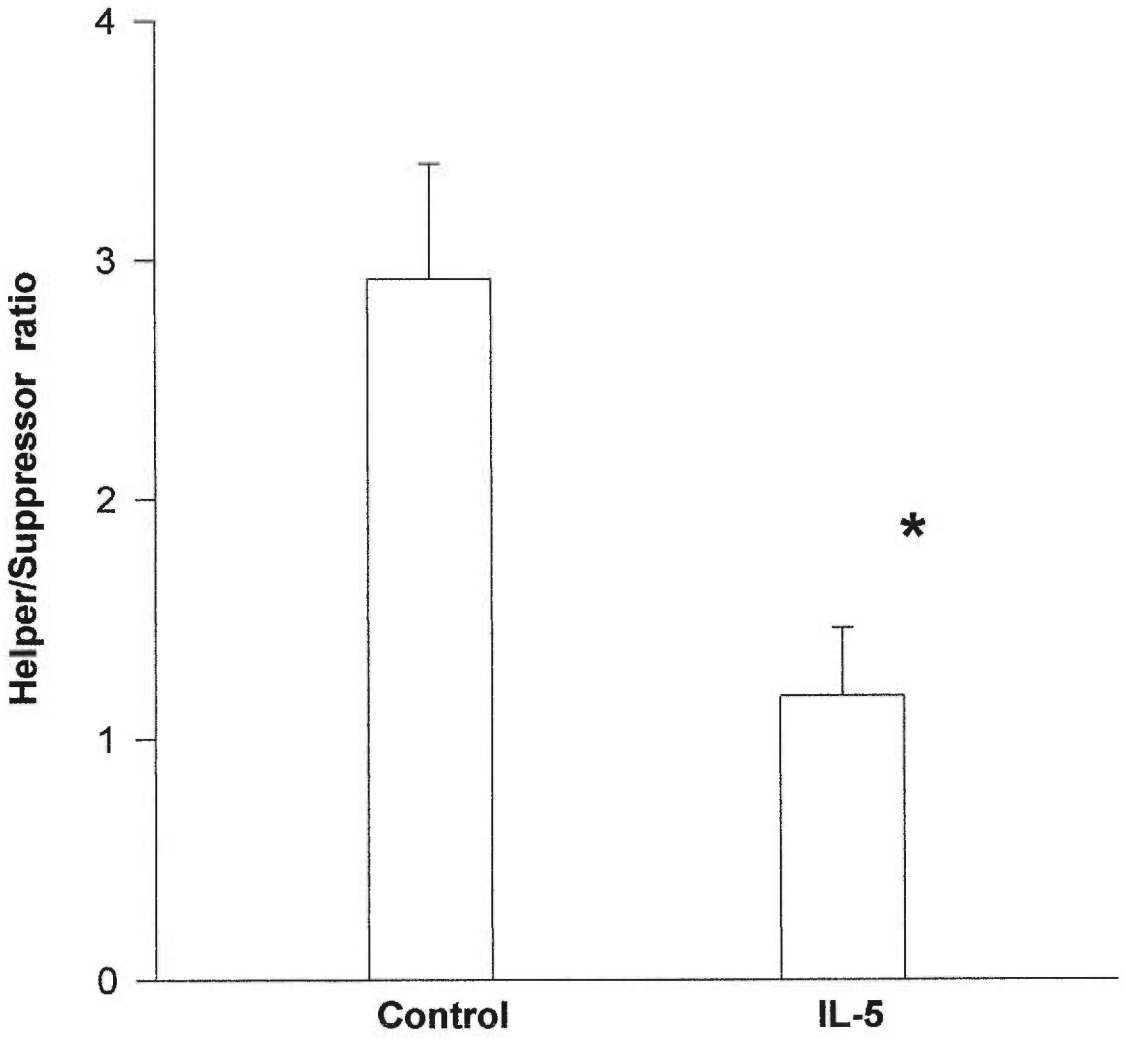

Table 1: Effect of dose of intra-tracheal rhIL-5 on the inflammatory cell populations from the bronchoalveolar lavage.

	Control (BSA/ 0μg IL-5) ($x10^6$)	10μg IL-5 ($x10^6$)
Total cells	6.65 ± 2.08	13.1 ± 3.07
Lymphocytes	0.154 ± 0.09	0.252 ± 0.067
Macrophages	3.95 ± 0.27	4.53 ± 0.92
Neutrophils	2.78 ± 0.73	8.01 ± 2.21*
Eosinophils	0.092 ± 0.07	0.243 ± 0.0403
Basophils	0.05 ± 0.03	0.0655 ± 0.0313

* $p<0.05$ between 10μg IL-5 and Control

Table 2: Effect of IL-5 on lung cytokine expression after ovalbumin challenge

	10μg IL-5 treated (positive cells/0.45 mm^2 basal mucosa)
Th1 cytokines	
IL-4	1.92 ± 0.28 *
IL-5	1.19 ± 0.08 *
Th2 cytokines	
IFN-γ	0.482 ± 0.042

* $p<0.05$ compared to IFN-γ level of positive cells

iscussion

L'objet de cette étude était d'évaluer chez le rat le rôle de l'IL-5 dans la réponse holinergique des voies respiratoire, leur inflammation ainsi que leur réaction après rovocation à l'OA. A l'administration intratrachéale de l'IL-5 on a constaté une augmentation e la réactivité bronchique à la méthacholine 20 heures après l'administration. Cette réponse a té associée à une augmentation significative de neutrophiles dans le lavage bronchoalvéolaire. e pré-traitement des rats avec l'IL-5 n'a eu aucun effet sur la réaction aigüe (RA) ou semi-etardeé (RSR) après provocation à l'OA mais a diminué le rapport des lymphocytes H/S du ang 8 heures après provocation antigénique et a augmenté l'expression de mARN de l'IL-4 et IL-5 au niveau des voies respiratoires et ceci 20 heures après la provocation. En outre, la ésistance pulmonaire a augmenté de manière significative chez des rats pré-traités à l'IL-5 et rovoqués à l'OA 20 heures après tandis que la réponse à la métacholine était inchangée.

La provocation à l'antigène chez les animaux sensibilisés se traduit par une activation es cellules dans les voies respiratoires et le recrutement d'une variété des cellules nflammatoires incluant les éosinophiles, les lymphocytes, les mastocytes et les neutrophiles 147,148,149). L'ensemble de ces cellules est impliqué dans les changements hysiopathologiques qui caratérisent l'asthme atopique (150). Bien que le mécanisme par equel ces changements se produisent ne sont pas clairement élucidés, des études récentes uggèrent que l'IL-5 pourrait jouer un rôle de modulateur important de l'inflammation llergique. Ces études avaient montré une augmentation de l'IL-5 dans le sang périphérique et es poumons des patients asthmatiques (146) et l'administration de cette cytokine à ces patients ugmente la réponse cholinergique et le nombre des éosinophiles activés dans les voies espiratoires (151). De plus, les études chez les animaux ont montré que l'administration de 'IL-5 aux cobayes sensibilisés provoque une hyperréactivité bronchique (HB) associée à 'antigène (152) et que les anticorps anti-IL-5 neutralisants empêchent la réponse semi-retardeé

ɔrès la provocation d'antigène (153). Bien que le rôle attribué à l'IL-5 est bien fondé, aucune ude n'a examiné les effets d'IL-5 chez les animaux qui ne présentent pas de réponses semi-tardé après sensibilisation et provocation à l'antigène. Nous avons réalisé cette étude avec la ɔuche RIJ des rats de BN qui ne développent pas de RSR après provocation à l'antigène (142) fin d'évaluer si l'IL-5 a la capacité de transformer les rats insensibles RIJ en ceux avec les aractéristiques présentes dans l'asthme atopique.

Nous avons débuté notre étude en déterminant si l'IL-5 recombinante humaine (rhIL-5) ɹui a une homologie de 71% avec l'IL-5 de rat) (154) était fonctionnelle chez le rats. Les xpériences effectuées sur les cellules mononuclées des rats ont montré que le rhIL-5 a induit ne différentiation des éosinophiles à partir des cellules progénitrices à des doses de 1 à 5 g/ml. Nous avons par la suite déterminé si l'administration intratrachéale de rhIL-5 a des ffets sur la réponse des voies respiratoires à la métacholine. Une augmentation de la réponse es voies respiratoires à la métacholine s'est produite 20 heures après l'administration ıtratrachéale de rhIL-5. Cependant, l'administration intratrachéale de rhIL-5 n'a aucun effet ur la réponse des voies respiratoires à la métacholine 30 minutes ou 72 heures après dministration. Ceci suggère que l'IL-5 agit indirectement. Les résultats obtenus à partir du avage bronchoalvéolaire plaident en faveur de cette hypothèse. En effet, nous avons trouvé ne augmentation proportionnele à la dose de l'IL-5 des cellules totales, et particulièrement des eutrophiles. Il est intéressant de constater que Lilly et ses collaborateurs (133) ont rapporté ne augmentation des éosinophiles et des neutrophiles dans le BAL des cobayes 24 heures près administration intratrachéale de rhIL-5. Par l'accumulation dans les poumons, les cellules nflammatoires recrutées peuvent libérer des médiateurs (leucotrienes, histamine, platelet-ctivating factor) qui ont la capacité d'augmenter l'HB (133). Une étude récente a montré une orrélation postive entre le nombre de cellules inflammatoires et de la sévérité du HB (146).

Dans l'étude présentée ici, on a constaté que l'IL-5 a causé une augmentation des

eutrophiles, mais pas des éosinophiles intrapulmonaires comme dans le cas du cobaye. Cette ifférence de résultats peut être dûe aux modèles animaux utilisés dans ces deux études. 'ependant, une raison technique ne peut être exclue non plus. La dégranulation et la relâche es médiateurs des éosinophiles a pu avoir lieu 20 heures après l'administration de l'IL-5, mpêchant ainsi leur mise en evidence par une coloration Wright-Giemsa. Cependant, nous vons obtenu les mêmes résultats en utilisant un immunomarquage grâce à un anticorps anti-MBP. Une autre raison est que peut-être les éosinophiles ne sont pas disponibles pour être ecrutées dans les poumons dans cette souche de rat. Jusqu'ici les expériences évaluant si l'IL-5 st impliqué dans l'asthme ont utilisé les modèles animaux qui développent des réponses hysiologiques après l'administration de l'antigène et ont prouvé que la réponse physiologique eut être augmentée par IL-5. Il est possible que dans les modèles "atopiques", les éosinophiles u leurs cellules souches soient aisément disponibles pour être recrutés dans les poumons ependant que chez les rats BN RIJ ils ne le sont pas. Bien que les éosinophiles soient connues our contenir les granules qui sont capables d'induire l'HB (155), l'étude mené par Lilly et ollaborateurs a indiqué que la présence des éosinophiles dans le tissu du poumon, n'est pas ujours suffisante pour modifier les réponses contractiles des voies respiratoires ce qui ggère que d'autres cellules ou médiateurs puissent être impliqués (133). Les neutrophiles bèrent des produits qui peuvent être impliqués dans les modifications de la fonction espiratoire en causant des dommages au tissu comme par exemple les radicaux de l'oxygène, rotéases, et protéines cationiques (156). Par conséquent, bien que la souche de RIJ du rat de N puisse ne pas répondre à IL-5 avec l'accumulation des éosinophiles, la dégranulation des eutrophiles peut être incriminée dans l'augmentation du l'HB à la métacholine.

En plus de son effet sur la réponse à la métacholine, l'IL-5 semble être impliquée dans ertaines réponses des voies respiratoires lors d'une provocation à l'antigène. Le prétraitement es cobayes avec l'IL-5 a montré une induction de l'éosinophilie et une bronchoconstriction

ɔrès provocation à l'antigène (157). De même, l'administration d'un anticorps monoclonal ɪti-IL-5 aux cobayes sensibilisés empêche l'influx des éosinophiles dans le BAL ainsi que HB après la provocation antigénique (14,134). Nous avons évalué les effets du pré-traitement vec le rhIL-5 sur la RA, la RSR, et l'HB des voies respiratoire chez les rats BN RIJ. 'administration intratrachéale de 3μg de rhIL-5, 24 heures avant le provocation antigénique, a pas affecté la RA, la RSR, ou l'influx cellulaire après provocation par rapport aux rats ɔntrôles ayant reçu la même quantité de BSA. Nos résultats differènt de ceux obtenus chez :s animaux qu'ont une prédisposition atopique. Il demeure possible que chez les rats rédisposés, l'IL-5 intratrachéal est capable d'augmenter les changements physiologiques après rovocation antigénique, mais que chez le rat BN RIJ les facteurs nécessaires pour développer ne réponse physiologique après le provocation antigénique néccessittent plus que l'IL-5. Bien u'ils n'ont pas été évalués dans cette étude, en plus de l'IL-5, la présence des IgE spécifiques l'allergène ainsi que certaines chémokines (éotaxine, MCP-4) ou d'autres cytokines (GM-'SF) semblent être nécessaires pour le développement de l'HB après provocation à l'allergène 158,133).

Puisque les changements physiologiques comme l'obstruction bronchiques et la réponse holinergique accrue des voies respiratoires qui se produisent après provocation à l'antigène, ous ont invité a examiner l'effet de l'IL-5 sur la résistance pulmonaire et la réponse des voies espiratoires 20 heures après la provocation à l'antigène. L'IL-5 a augmenté la résistance ulmonaire 20 heures après le provocation à l'antigène, mais n'a eu aucun effet sur la réponse es voies respiratoires au métacholine. L'augmentation de la résistance des voies respiratoires eut être dû à plusieurs facteurs dont de plus de lymphocytes T qui produisent l'IL-4, la ynthèse accrue d'IgE et la relâche des médiateurs par les cellules effectrices autre que les osinophiles (159). Dans notre étude, nous avons trouvé une diminution significative du apport des cellules T helper/suppressor dans le sang chez les rats ayant reçu l'IL-5 par rapport

ıx animaux contrôles 8 heures après provocation antigénique. Cette différence est rincipalement dûe à une augmentation en cellules de $CD8^+$/suppressor dans le sang ériphérique des animaux provoqués et non à une diminution de cellules CD4 dans le sang. ien qu'il y ait une dimunition relative des lymphocytes T suppressor dans les patients résentant la rhinitis allergique (160.161), la dermatite atopique (162) et l'asthme allergique 163), une diminution du rapport helper/suppressor dans le sang a été rapportée au cours de la SR chez l'homme (3). Il a été suggére que les lymphocytes T helper sont recruitées dans les ronchi chez les sujets qui développent une RSR car l'on a démontré une augmentation des ellules $CD4^+$ dans le lavage bronchoalvéolaire après provocation antigénique (164). Il est ıtéressant de constater que l'IL-5 induit le mêmes changement du rapport $CD4^+/CD8^+$ après la rovocation allergénique que chez les rats BN au cours d'une RSR. L'absence d'une RSR chez s rats traités avec l'IL-5 malgré les changements du rapport $CD4^+/CD8^+$ étudiés suggère que s changements en sous types lymphocytaires n'ont pas de rôle dans la réaction physiologique SR.

Le traitement avec l'IL-5 avant la provocation antigénique a causé une augmentation de ı R_L 20 heures après la provocation avec l'OA. Le mécanisme de ces changements ne sont as clair mais une augmentation Th2 du cytokine mRNA peut en être la cause. La production e ces cytokines peut agir sur différentes voies pendant une réaction allergique. L'IL-4 favorise interaction entre la molécule d'adhérence VCAM-1 sur les cellules endothéliales et les ıtégrines α-4 sur les leucocytes menant au recrutement sélectif des éosinophiles et des mphocytes dans les tissus ou se produisent une réaction allergique (165). L'IL-4 cause une ugmentation modérée de l'expression de VCAM-1 sur les cellules endothéliales, cependant, uand le TNF-alpha et l'IL-4 sont combinés, il y a une augmentation synergique dans expression du VCAM-1 et un prolongation importante de la durée de l'expression de VCAM- sur la surface des cellules (166). De plus, on a montré que la production des IgE par les

ellules B est IL-4-dépendante (167). L'IL-4 induit la différenciation des mastocytes (168) qui euvent non seulement libérer l'histamine et d'autres enzymes capables d'endommager épithélium des voies respiratoires (169), mais qui peuvent a leur tour libérer l'IL-4 et l'IL-5 .70). L'IL-5 est impliqué dans le recrutement et l'activation des éosinophiles ainsi que leur dhérence aux cellules endothéliales (171,172) et induit également sélectivement le égranulation sélective des éosinophiles et la cytotoxicité dépendante des anticorps (173).

Il a été démontré que l'administration de l'IL-5 suivie d'une provocation bronchique à antigène augmente le nombres d'éosinophiles dans le tissu pulmonaire ainsi que l'HB chez s cobayes (158). Cependant, dans notre étude il n'y avait pas d'augmentation de l'HB après dministration de l'IL-5 et la provocation à l'OA chez les rats BN. Il est possible que augmentation de la R_L chez les rats pré-traités par l'IL-5 ait masqué les effets sur l'HB. Il est robable que d'autres facteurs soient nécessaires pour avoir une HB chez les rats BN RIJ. Les acteurs tels que l'augmentation des cellules $CD4^+$, des niveaux plus élevés d'IgE, la présence es éosinophiles, de TNF-alpha, de GM-CSF, et des chémokines tels que l'éotaxine et le ANTES peuvent être nécessaires pour permettre une augmentation de l'HB. En outre, ertaines caractéristiques des cellules epithéliales et des cellules musculaires lisses peuvent galement faire défaut chez le rat BN RIJ pour que l'HB ait lieu après une provocation ntigénique.

En conclusion, nous avons trouvé que l'administration intratrachéal de rhIL-5 à des rats ui n'ont pas les caractéristiques de l'asthme atopique a causé quelques changements ellulaires et physiologiques que l'on retrouve dans l'asthme atopique. Les stratégies nérapeutiques dirigé contre l'IL-5 seule peuvent être insuffisantes pour retourner la réponse es voies respiratoires à la normale chez les patients asthmatiques atopiques.

iscussion

This study assessed whether rhIL-5 could directly affect cholinergic airway :sponsiveness, airway inflammation, and the airway response after OA challenge in normal its. Intratracheal administration of rhIL-5 increased airway responsiveness to Mch 20 hours ıter, but had no effects 30 minutes or 72 hours after administration. The increased airway :sponsiveness to métacholine was accompanied by a significant increase in total cells as well s in neutrophils in lung lavage. Pretreatment of rats with 3µg of rhIL-5 did not affect the ER or R after OA challenge but decreased the blood H/S ratio 8 hours after challenge and increased -4 and IL-5 lung mRNA expression 20 hours after challenge. In addition, baseline lung :sistance increased significantly in IL-5 pretreated/challenged rats 20 hours after OA hallenge while Mch responsiveness was unchanged.

Allergen challenge in sensitized animals causes the activation and recruitment into the irways of a variety of cells, including eosinophils, lymphocytes, mast cells, and neutrophils 147,148,149). These inflammatory cells are involved in the physiological and pathological hanges that are present in atopique asthma (150). The mechanism by which these changes ccur are not clearly identified, yet recent studies suggest that IL-5 may be an important ıodulator of allergic inflammation. Studies to date have shown IL-5 to be increased in the lood and lungs of patients with asthma (146) and that administration of IL-5 to patients with llergic bronchial asthma increases cholinergic responsiveness and the number of activated osinophils in the airways (151). Studies in animals have shown that administration of IL-5 to :nsitized guinea pigs enhances antigen-induced hyperresponsiveness (152) and that IL-5 eutralizing antibodies inhibit the late phase response after antigen challenge (153). Although ıere is convincing evidence that IL-5 is important in asthma and in animal models of asthma, o studies have examined the effects of IL-5 in animals that do not respond to sensitization and ntigen challenge with late-phase responses. We performed this study in the RIJ strain of BN

ats that do not develop LR after antigen challenge (142) in order to assess whether IL-5 has the apacity to transform unresponsive RIJ BN rats into those with the characteristics encountered n atopique asthma.

We first determined whether rhIL-5 (which has 71% homology with rat IL-5 (154)) was ffective in rats in vitro. Experiments performed on rat mononuclear cells showed that rhIL-5 aused a concentration-dependent proliferation of eosinophils from progenitors at a dose anging from 1 to 5 ng/ml. We then determined whether intratracheal administration of rhIL-5 ad effects on airway responsiveness to Mch. An increase in airway responsiveness to Mch ccurred 20 hours after intratracheal administration of rhIL-5 at a dose exceeding 3 µg. ecombinant human (rh)IL-5 did not affect airway responsiveness 30 minutes or 72 hours after ntratracheal administration. The time course of these effects would suggest that IL-5 is acting ndirectly. Results obtained from lung lavage would concur with this hypothesis. Indeed, we ound a dose-dependent increase in the total cellular return, which consisted mainly in eutrophils, from lung lavage. Interestingly, Lilly et al (133) reported a dose-dependent ncrease in the recovery of eosinophils and neutrophils from the BAL fluid of guinea pigs 24 ours after intratracheal administration of rhIL-5. By accumulating into the lungs, activated nflammatory cells may release mediators (e.g. leukotrienes, histamine, platelet-activating actor) that have the ability to augment airway hyperresponsivess (133). A recent study has hown a correlation between the presence of increased inflammatory cells and the severity of irway hyperresponsiveness (146).

In this study we found that IL-5 caused an increase in BAL neutrophils, but not in osinophils as has been described in guinea pigs. This discrepancy may be due to the inherent ifferences between the two animal models. BN RIJ rats may not have had an increased umber of eosinophils in lung lavage because of technical reasons. Eosinophil degranulation nd mediator release may have already taken place 20 hours following IL-5 administration

reventing us from measuring their presence by a regular Wright-Giemsa stain. However, we btained the same results when we employed MBP staining which adds more probability to the onclusion that eosinophils were not increased in lung lavage 20 hours after IL-5 dministration. Another reason for this discrepancy is that perhaps eosinophils are unavailable or recruitment into the lungs in this strain of rat. Until now experiments assessing whether IL- is involved in asthma have employed animal models that develop these responses after ntigen challenge and shown that the physiological response can be increased by IL-5. It is ossible that in these animals, eosinophils or their progenitors are readily available to be ecruited into the lungs whereas in BN RIJ rats they are not. Although eosinophils are known to ontain granules that are capable of inducing airway hyperresponsiveness (155), a study by illy et al reported that the presence of eosinophils in lung tissue, by itself, is not always ufficient to alter airway contractile responses which suggests that other cells or mediators may e involved (133). Neutrophils release products that have the potential for altering airway unction by causing tissue injury: oxygen radicals, proteases, and cationic proteins (156). 'herefore, although the RIJ strain of BN rat may not respond to IL-5 with increased eosinophil ccumulation in the airways, AHR to Mch may have still occurred through neutrophil egranulation.

In addition to affecting cholinergic airway responsiveness, IL-5 seems to be involved in ertain aspects of the airway response that occur after antigen challenge. Pretreatment of uinea pigs with IL-5 has been shown to induce lung eosinophilia and bronchoconstriction after ntigen challenge (157). As well, administration of an monoclonal anti-IL-5 antibody to ensitized guinea pigs inhibits BAL eosinophilia and airway responsiveness after OA challenge 14,134). We assessed the effects of pretreatment with rhIL-5 on the ER, LR, and airway yperresponsiveness in RIJ BN rats that do not have a physiological response after sensitization nd Ag challenge. Intratracheal administration of 3μg of rhIL-5, 24 hours prior to OA

hallenge, did not affect the ER, LR, or cellular influx after challenge when compared to ontrols receiving the same amount of BSA. It may be that in the predisposed subject, ıtratracheal IL-5 is capable of increasing all the changes that occur after antigen challenge, but ıat in the normal rat several crucial factors that are necessary for an atopique airway response ıay be lacking. Although not assessed here, these include the presence of increased allergen-pecific IgE which is necessary with IL-5 for the development of AHR following allergen hallenge (158), the presence of certain chemokines (eotaxin, MCP-4) or of other cytokines GM-CSF) (133).

Since the physiological changes that occur after antigen challenge also include airway bstruction and increased cholinergic airway responsiveness, we examined the effect of IL-5 on ıng resistance and airway responsiveness 20 hours after antigen challenge. IL-5 increased lung esistance in rats 20 hours after antigen challenge, but had no effect on airway responsiveness) métacholine. The changes in airway resistance can be linked to various immunological vents including T cell activation, IL-4 production, IgE synthesis and mediator release by ffector cells such as eosinophils and mast cells (159). In our study, we found a significant ecrease in the helper/suppressor T cell ratio in the blood between IL-5 treated/challenged and ontrol animals 8 hours after antigen challenge. The difference is mainly due to an increase in $CD8^+$/suppressor cells in the blood of challenged animals. Studies have shown a relative eficiency of suppressor T lymphocytes in patients with allergic rhinitis (160,161), atopique ermatitis (162) and allergic asthma (163). A decrease in the helper/suppressor ratio in the lood has been described during the LR in humans (3). It has been postulated that $CD4^+$ mphocytes are recruited into the lungs after antigen challenge in the subjects that develop a R. This hypothesis has been predicated upon the finding of increased $CD4^+$ cells in lung ıvage after antigen challenge (164). Although we have previously reported a decrease in the elper/suppressor ratio in the blood of rats that develop a LR, we have not found an increase in

$CD4^+$ cells in the lung tissue of rats that develop a LR (3). Interestingly, pretreatment with IL-5 nduced the same changes in the $CD4^+/CD8^+$ ratio as that previously reported in BN rats with .R. The absence of a LR in the rats studied here would suggest that the changes in lymphocyte ubsets reported during a LR are not involved in the occurrence of the LR.

Pretreatment with IL-5 prior to antigen challenge caused an increase in R_L 20 hours fter antigen challenge. The mechanism of these changes is not clear but an increase in Th2 ytokine mRNA may have been related to the increase in R_L. The increased production of nese cytokines may act on different pathways during an allergic reaction. IL-4 promotes the nteraction between vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells and lpha 4 integrins on leukocytes leading to the selective recruitment of eosinophils and ymphocytes in allergic diseases (165). IL-4 causes a moderate increase of VCAM-1 expression n endothelial cells, however, when TNF-alpha and IL-4 were combined, there was a ynergistic increase in VCAM-1 expression and a dramatic prolongation of the appearance of /CAM-1 on the cell surface (166). As well, it has been shown that B cell production of IgE epends on IL-4 (167). IL-4 increases the growth of mast cells (168) which may not only elease histamine and other enzymes capable of damaging the airway epithelium (169), but also elease IL-4 and IL-5 (170). IL-5 is involved in the recruitment and priming of eosinophils and nhances adhesion of eosinophils to endothelial cells (171,172). IL-5 also selectively induces osinophil degranulation and antibody-dependent cytotoxicity (173).

IL-5 administration followed by allergen challenge has been shown to increase osinophil numbers in lung tissue and increase AHR (158). However, in our study there was no bserved increase in AHR to métacholine following IL-5 treatment/OA challenge. It is possible nat the increased R_L in IL-5 pre-treated rats masked the effects on AHR. Perhaps other factors re necessary for AHR to occur after antigen challenge in the BN RIJ rat. Factors such as ncreased $CD4^+$ T cell activation, higher IgE levels, the presence of more eosinophils,

rtokines such as TNF-alpha, GM-CSF, immunoglobulins, and chemokines such as eotaxin and ANTES may be necessary for AHR to occur. In addition, certain characteristics of cells that nstitute normal lung tissue i.e. epithelial cells and smooth muscle cells may also be lacking the BN RIJ rat for AHR to occur after antigen challenge.

In conclusion, we have found that intratracheal administration of rhIL-5 to rats that do ot have the characteristics of atopique asthma will cause some but not all of the cellular and ysiological changes that are found in atopique asthma. Therapeutic strategies directed gainst IL-5 alone may be insufficient to revert the airway response to normal in atopique thmatics.

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Acknowledgment

I would like to thank the following people for their help and support on this project. My parents for their unyielding and sometimes relentless support. The following people provided important guidance in the laboratory: Maria Makroyanni, Li-Jing Xu, , Rosa Pantano, Jamilah Saeed, Serge Séguin, Zoulfia Allakaverdi, and Samer Al-Assaad. As well, I am grateful to Dr. Bouchaib Lamkhioued for his invaluable assistance in technical writing. Finally, I would like to recognize my supervisor, Dr. Paolo Renzi for his great effort in realizing this project and encouraging me throughout the course of the study.