ALLERGEN SPECIFIC CYTOKINE PRODUCTION BY CELLS DERIVED FROM HUMAN NASAL POLYPS

Narinder Singh

A thesis submitted in fulfilment of the requirements for the degree of

Master of Surgery

Faculty of Medicine

University of Sydney

ABSTRACT

Background

Traditionally, the clinical response to allergen exposure in subjects with atopic diseases, including atopic asthma and allergic rhinitis, has been thought to be mediated by a Th2-type cytokine profile. Conversely, the lack of clinical response to allergen in non-atopic subjects has been thought to reflect either no immunological activity or a Th1-type response. Recent murine models have suggested a more complex interaction between Th1 and Th2 responses, with a critical functional role for IL-10 secreting regulatory T cells in suppressing allergic airway inflammation. There is a paucity of corresponding data regarding human airway cells.

Objectives

The aims of this thesis were to study the *ex vivo* allergen-specific cytokine responses of cells derived from human nasal polyps. We hypothesised that IL-10 producing regulatory T cells have a critical functional role in suppressing allergen-specific Th2 and/or Th1 responses in respiratory cells derived from human nasal polyps.

Materials and methods

Nasal polyp tissue was harvested from 20 non-smoking adults with nasal polyposis requiring surgery. Atopic status was determined by skin prick testing. Following isolation by matrix proteolysis, cell suspensions were incubated with allergen (cat, grass or house dust mite) for 6 days. Cytokine production was determined by cytometric bead array.

Results

Allergen stimulation of human nasal polyp cell suspensions significantly enhanced production of IL-10, but not IL-5 or IFN-γ. Under the same conditions, neutralisation of IL-10 significantly increased allergen-specific IL-5,

IFN-γ and TNF-α production. Cell depletion experiments demonstrated the observed IL-10 was either derived from T cells or via a T cell dependent process. Subsequent Intracellular cytokine staining experiments demonstrated T cells immunoreactive for IL-10, but not IL-2 or IL-13.

Conclusions

Allergen-specific regulatory T cells play a critical functional role in human respiratory tissue derived from nasal polyps by regulating abnormal Th2 and Th1 responses to common inhaled aeroallergens, through mechanisms dependent on allergen-specific production of IL-10.

PREFACE

This thesis records experimental work performed by the candidate in the laboratory of The Randall division of Cell and Molecular Biophysics, King's College, London, UK and Guy's Hospital, Guy's and St Thomas' NHS Foundation Trust, London, UK.

The work was performed under the supervision of:

Primary Supervisor

Professor William Peter Rea Gibson (Sydney)

Associate Supervisors

Dr Alex Faith (London)

Professor Catherine Hawrylowicz (London)

Professor Christopher Corrigan (London)

Professor Hannah Gould (London)

Professor James May (Sydney)

ACKNOWLEDGEMENTS

This thesis could not have been completed without the patience, skills and encouragement of the following people:

Dr Alexander Faith

Professor Christopher Corrigan

Professor Catherine Hawrylowicz

David Richards

Haw Lu

Dr Sarah Dimeloe

Dr Belinda Butcher

Dr John Brannan

Mr David Roberts

Ms Elfy Chevretton

Dr Sophie Farooque

Professor Tak Lee

Professor William Peter Rea Gibson

Professor Hannah Gould

Professor James May

FUNDING

This thesis could not have been completed without the generous support of the following funding bodies and research grants:

1. **The Garnett Passe and Rodney Williams Memorial Foundation Grant-in-aid**
2. **The John Brooke Moore Research Scholarship in Surgery (University of Sydney)**
3. **The Vernon Barling Memorial Fellowship (University of Sydney)**

DEDICATION

Dedicated to my parents and possums.

LIST OF PUBLICATIONS

Singh N, Roberts D, Chevretton E, Corrigan C, Hawrylowicz C, Faith A. Allergen specific cytokine production by cells derived from human nasal polyps. Internal Medicine Journal 2008; 38 (Suppl. 6): A149–A176

Faith A, Singh N, Chevretton E, Roberts D, Lee T, Corrigan C, Hawrylowicz C. Counter regulation of the high affinity IgE receptor, FcεRI, on human airway dendritic cells by IL-4 and IL-10. Allergy. 2009 Nov; 64(11):1602-1607.

Faith A, Singh N, Chevreton E, Roberts D, Lee TH, Corrigan C, Hawrylowicz C. Expression and function of the high affinity IgE receptor, FcεRI, on human airway dendritic cells: S10. Clinical & Experimental Allergy. 38(12):1981, December 2008.

Faith A, Singh N, Chevreton E, Lee TH, Hawrylowicz C. The high affinity IgE receptor (FcεRI) is expressed by human respiratory tract dendritic cells and is susceptible to modulation by inflammatory cytokines: S15. Clinical & Experimental Allergy. 37(12):1885, December 2007.

Faith A, Singh N, Farooque S, Dimeloe S, Richards D, Lu H, Roberts D Chevretton E, Lee T, Corrigan C, Hawrylowicz C. T cells producing the anti-inflammatory cytokine IL-10 regulate allergen-specific Th2 responses in human airways. Allergy. 2012 Aug; 67(8):1007-1013.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AFS	Allergic fungal sinusitis
AHR	Airway hyperreactivity
APC	Antigen Presenting Cell
AR	Allergic rhinitis
ARIA	Allergic Rhinitis and its Impact on Asthma
B7RP-1	B7-related protein-1
CBA	Cytometric bead array
CRS	Chronic rhinosinusitis
CRSsNP	Chronic rhinosinusitis without nasal polyposis
CRSwNP	Chronic rhinosinusitis with nasal polyps
CTLA-4	Cytotoxic T-lymphocyte antigen 4
DC	Dendritic cell
ECP	Eosinophil cationic protein
ELISA	Enzyme-linked immunosorbent assay
EMCRS	Eosinophilic mucin rhinosinusits
EPO	Eosinophil peroxidase
FCS	Foetal calf serum
FoxP3	Forkhead box P3
GINA	Global Initiative for Asthma

GM-CSF	Granulocyte-Macrophage Colony-Stimulation Factor
HBSS	Hank's Buffered Saline Solution
HDM	House dust mite
ICOS	Inducible co-stimulator
IFN-γ	Interferon gamma
IgE	Immunoglobulin E
IL	Interleukin
Jak	Janus Kinase
LPS	Lipopolysaccharide
MBP	Major basic protein
MHC	Major histocompatibility complex
MMPs	Matrix metalloproteinases
NAR	Non-allergic rhinitis
NP	Nasal polyposis
nTreg	Natural T regulatory cell
PAMP	Pathogen-associated molecular pattern
PD-1	Programmed death-1
PDGF	Platelet Derived Growth Factor
PGE2	Prostaglandin E2
PPD	Purified protein derivative
RANTES	Regulated on Activation Normal T Expressed and Secreted

ROR	Retinoid orphan nuclear receptor
RPMI	Roswell Park Memorial Institute
RT-PCR	Reverse transcription polymerase chain reaction
SAE	*Staphylococcus Aureus* enterotoxin
STAT	Signal transducer and activator of transcription
TCR	T cell receptor
TGF- β	Transforming Growth Factor Beta
Th	T helper cell
TLR	Toll-like receptors
TNF-α	Tumour necrosis factor alpha
Treg	T regulatory cell
TSLP	Thymic stromal lymphopoietin
Tyk	Tyrosine Kinase
VEGF	Vascular Endothelial Growth Factor

UNITS

cm	centimetre
µm	micrometre
mg	milligrams
µg	micrograms
pg	picograms
L	litre
mL	millilitre
L/min	litres per minute
yr	year
hr	hour
min	minutes
sec	second
^{o}C	degrees Celcius

STATISTICS

ANOVA	Analysis of variance using repeated measures
Gmean	geometric mean
ICC	Intra-class correlation coefficient
rp	Pearson's correlation coefficient
rs	Spearman's Rank correlation coefficient
SD	standard deviation
SEM	standard error of the mean
95%CI	95% confidence interval

1 INTRODUCTION

1.1 Allergic Rhinitis

1.1.1 Definition

Allergic rhinitis (AR) is clinically defined as allergen-associated, IgE-mediated inflammation of the nasal membranes (1, 2). It is characterised by symptoms including rhinorrhoea, nasal congestion, sneezing and nasal/ palatal itching, along with postnasal drainage and ocular symptoms (itchy, watery eyes) (1).

1.1.2 Epidemiology

The prevalence of AR is between 10-30% of adults and up to 40% of children (3-5), particularly those between the ages of 6-14 years (1, 6, 7). These figures are based on physician-diagnosis. The actual prevalence may be greater as up to one third of individuals with AR never see a physician, preferring to self-medicate or borrow medications from friends and family (8). AR has been identified as one of the top ten reasons for visiting a primary care clinic (9).

Both the incidence and severity of AR appears to be increasing in modern urban areas (6, 10). European studies report an increase in prevalence of up to 3.5% per decade (11). Possible contributing factors for this change include the effects of environmental and lifestyle changes, such as air pollution, indoor environment, affluence, exposure to new allergens and a psychologically stressful lifestyle (10, 12).

Increases in the incidence of childhood AR in “Westernised” urban areas have been attributed to the “Hygiene Hypothesis” which correlates a modern urban upbringing with a low exposure to microbial fragments (12, 13).

1.1.2.1 Risk Factors

AR is related to other atopic diseases, including asthma and eczema (1). Proposed risk factors include genetics and family history, early life risk factors, ethnicity, allergen exposure, rural-urban differences, and smoking (2).

There appears to be a genetic component with the most well-established risk factor for AR being a family history of allergy, especially AR (1, 2).

Outdoor allergens appear to pose the greatest risk for seasonal rhinitis compared to indoor allergens (1, 2). Early exposure to allergens may be protective, however, this hypothesis needs further testing (1).

AR is more common in urban areas. This may be due to the higher concentration of pollutants in urban areas, which are thought to increase the allergenic potency of pollens (1). Other hypotheses relate to early protective allergen exposure in rural areas (2).

Existing literature on the influence of early life risk factors (such as young maternal age) is not clear. Evidence regarding ethnicity and smoking is contradictory and further research into these areas is required (1, 2).

1.1.3 Pathophysiology and Aetiology

1.1.3.1 Sensitisation

The initial sensitisation process in AR is known as the primary immune response. Subsequent and ongoing reactions to allergen are known as the secondary immune response.

Primary Immune Response

The primary immune response generally occurs in childhood, although occupational and other forms of AR can result from sensitisation later in life.

The primary immune response is mediated by allergen interaction with antigen presenting cells (APC), of which the most potent is the dendritic cell

(DC) (14). The typical result of the interaction between harmless antigen and DCs is tolerance, whilst the result of the interaction between pathogenic antigens and DCs is immunity. However, under certain circumstances, harmless antigens may trigger a T helper 2 type (Th2) response, resulting in allergy. A Th2-type immune response will be mounted if DCs reach full maturity during contact with an antigen. This can occur in the presence of concomitant inflammatory stimuli such as lipopolysaccharide (LPS) or respiratory infections. Recent evidence suggests that mast cells may play a role in initiating DC maturation in the presence of endotoxins by releasing tumour necrosis factor alpha (TNF-α) (15, 16). DC maturation may also occur through contact with certain antigens that have the capacity to induce DC maturation directly. Der p 1, which is derived from the house dust mite *Dermatophagoides pteronyssinus*, directly induces DC maturation through enzymatic activity, leading to a Th2-type allergic response (17, 18).

Once primary sensitisation has occurred, subsequent allergen exposure will lead to secondary immune responses. These comprise the typical episodes of AR seen clinically.

Secondary Immune Response

The secondary immune response of AR has both early and late phase components.

Secondary Immune Response – Early Phase

The early phase commences within minutes of allergen exposure and subsides within 60 minutes (19). It is characterized by sneezing, rhinorrhoea, nasal obstruction and pruritis (20). Cross-linking of IgE by allergen triggers a complex local and systemic cascade of events. Activation of mast cells and basophils results in the immediate release of preformed histamine and granule proteins such as tryptase. Leukotrienes are released through activation of membrane phospholipids. Cytokines are released pre-formed from mast cells and are produced by T cells. These include Th2-type

cytokines such as Interleukin-4 (IL-4), IL-5 and IL-13 and inflammatory cytokines such as IL-6, IL-8, IL-10 and TNF-α (21-23).

Secondary Immune Response – Late Phase

The late phase reaction typically occurs 4 to 5 hours following allergen exposure in around 30-40% of patients (24). It peaks at 6 to 8 hours and subsides 12 to 24 hours after allergen challenge (19). Nasal obstruction is the main symptom. Inflammatory cells, such as eosinophils, are recruited and activated by mediators from the early phase reaction along with other cytokines and chemokines (25), particularly IL-5 (26).

During the secondary immune response, antigen presentation to T-cells can be performed by both DCs and any other cell expressing the major histocompatibility complex (MHC) class II (1).

Non IgE mediated

Recent evidence has demonstrated that certain allergens are able to bypass immunoglobulin E (IgE) binding and activate cells directly via the “innate” immune response (27). House dust mite allergens have been shown to activate epithelial cells in vitro (28) inducing pro-inflammatory, pro-Th2 cytokine and chemokine release (29, 30). The relative importance of this non-IgE mediated mechanism is yet to be determined.

1.1.4 Classification

1.1.4.1 Traditional classification

AR has traditionally been classified into seasonal and perennial variants (2). Seasonal AR is associated with allergens that are present in greater quantities during defined periods of the year. Typically, these include outdoor allergens such as grass and tree pollens. Perennial AR occurs throughout the year and is associated with ubiquitous year-round allergens such as house dust mite allergens, indoor moulds, cockroaches and animal dander (1, 2).

Due to the non-specific nature of its symptoms, perennial AR can often be difficult to identify clearly. It may occur simultaneously or be confused with chronic rhinosinusitis, recurring respiratory infection, nasal polyposis and non-allergic vasomotor rhinitis. Isolated seasonal AR, however, is more readily identified (10).

1.1.4.2 ARIA classification

Despite this traditional classification system, in practice, most patients with AR have been demonstrated to have a mixed aetiology, involving sensitisation to more than one allergen (31, 32). Indeed, a European study demonstrated that only around 20% of all patients are sensitised to a single trigger, compared with around 40% of patients sensitised to at least five triggers and 10–15% of patients sensitised to two to four triggers (33). Furthermore, many individuals sensitised to seasonal allergens exhibit persistent symptoms year round, whilst many other individuals sensitised to perennial allergens experience symptoms only intermittently (31, 32). As a consequence, the Allergic Rhinitis and its Impact on Asthma group (ARIA) proposed a new classification system in 2001, dividing patients into *intermittent* and *persistent* groups along with *mild* and *moderate-severe* variants (Figure 1.1.1) (1). This classification system has been validated through cross-sectional and multi-centre studies (31, 33, 34).

Intermittent

Symptoms are present:

- Less than 4 days a week
- OR, for less than 4 weeks

Persistent

Symptoms are present:

- More than 4 days a week
- AND, for more than 4 weeks

Mild

None of the following are present:

- Sleep disturbance
- Impairment of daily activities, leisure and/or sport
- Impairment of school or work
- Troublesome symptoms

Moderate-Severe

One or more of the following are present:

- Sleep disturbance
- Impairment of daily activities, leisure and/or sport
- Impairment of school or work
- Troublesome symptoms

Figure 1.1.1: ARIA classification of allergic rhinitis (1)

1.1.5 Clinical Features

AR is characterised by nasal symptoms (including rhinorrhoea, sneezing, nasal blockage, itching, post-nasal drip) and ocular symptoms (itchy, watery eyes) following allergen exposure (2). Symptoms are spontaneously reversible or reversible with treatment (2).

1.1.6 Impact and burden

AR has been demonstrated to have significant morbidity, affecting quality of life, productivity at work, concentration, learning ability (in children), sleep, mood and daily activities (2, 35-38). AR is associated with other conditions including sinusitis, asthma and otitis media (1, 39-42). A 1996 study estimated the total annual cost of AR in the United States to be $US6 billion (43).

1.1.7 Differentiation from non-allergic rhinitis

Non-allergic or “vasomotor” rhinitis (NAR), despite resulting in similar symptoms as AR, is a markedly different disease. As in AR, NAR is characterised by nasal congestion and rhinorrhoea. Associated symptoms can include post nasal drip, throat clearing, cough, eustachian tube dysfunction, sneezing, hypersomnia, facial pressure or headache and, generally, no nasal, pharyngeal or ocular itching (44).

Diagnosis is based upon symptoms and the absence of specific IgE responses by skin, serologic, or entopy (nasal mucosal challenge) testing.

The pathophysiology of NAR is poorly understood. NAR is thought to include several conditions, with the key area of interest involving hyperresponsiveness of C-fibre sensory nerves without nasal mucosa inflammatory changes (45).

1.2 Asthma

1.2.1 Definition

The Global Initiative for Asthma (GINA) guidelines define asthma as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role (46). The airway inflammation in asthma is associated with bronchial hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing (46, 47).

1.2.2 Epidemiology

The prevalence of asthma can vary depending upon geographical location. However, it is estimated that asthma affects 300 million people worldwide (48). Along with AR, the overall prevalence of asthma appears to be increasing in western nations, particularly in urban areas. In children, asthma prevalence and severity has increased over recent decades (3).

Asthma prevalence in Australia varies between 14 to 16% in children and 10 to 12% of adults (47).

1.2.2.1 Risk factors

Risk factors include family history, atopy and AR (49), early respiratory viral infections (50), prematurity, air pollution, exposure to cigarette smoke (51) and obesity (52).

1.2.3 Pathophysiology

The 3 hallmarks of asthma are airway inflammation, reversible airway obstruction and bronchial hyperreactivity (53). Chronic inflammation of the bronchial mucosa leads to mucosal oedema, vasodilation, cellular infiltration, epithelial injury, smooth muscle and mucous gland hypertrophy and eventually, basement membrane thickening and fibrosis (53).

Mast cells are a key feature of asthma and are located in the airway tissue with some evidence that they may infiltrate airway smooth muscle (54). Cells

that infiltrate the airway tissue and enter the lumen are detectable in induced sputum and have recently been used to describe varying inflammatory phenotypes in asthma (55). Four distinct phenotypes have been identified: eosinophilic, neutrophilic, mixed inflammatory and paucigranulocytic (55).

1.2.4 Clinical Features

Clinically, asthma is associated with symptoms of wheeze, chest tightness, shortness of breath and cough (47). Symptoms are typically recurrent or seasonal, worse at night or in the early morning, have obvious triggers and are relieved by short acting bronchodilators (47).

Objective measures of airway hyperresponsiveness, including spirometry and formal lung function testing are useful in diagnosing and monitoring the disease.

1.2.5 Impact and burden

Asthma represents a considerable burden in terms of morbidity and economic impact. Ongoing care requires frequent monitoring and acute exacerbations account for numerous emergency department visits. In the United States, in 2007, the total incremental cost of asthma to society was estimated at $US56 billion, with productivity losses due to morbidity accounting for $US3.8 billion and productivity losses due to mortality accounting for $US2.1 billion (56). Mortality rates appear to be declining from the high rates seen in the 1980's. However, as deaths from asthma are largely preventable, the mortality rate is still concerning at around 1.5 deaths per annum for every 100,000 individuals in the United States (57).

1.3 The Unified Airway

1.3.1 Definition

Epidemiological evidence and patterns of disease manifestation implicate a link between AR and atopic asthma, suggesting that both conditions are

manifestations of a common pathological process. This has led to the concept of the “Unified airway” (58).

1.3.2 Allergic Rhinitis is a risk factor for Asthma

Numerous studies have found a correlation between rhinitis (allergic and non-allergic) and asthma. Around one third of patients with rhinitis (AR and NAR) will have concurrent asthma (59). In AR, this figure increases to around 40% (59). Long-term follow-up studies have demonstrated that a high proportion of patients with rhinitis alone will subsequently proceed to develop asthma over time (49, 60-62). In fact, rhinitis is considered as an independent risk factor for the development of asthma (49, 63).

AR is a predictor of bronchial hyperreactivity, even in patients without a diagnosis of asthma (64). In patients with rhinitis, positive skin prick tests to inhalant allergen, particularly house dust mite (HDM), is a significant risk factor for asthma (65).

1.3.3 Asthma is a risk factor for Allergic Rhinitis

Conversely, it has been reported that symptoms of AR are experienced by between 30-99% of persons with asthma (66).

1.3.4 Asthma is associated with nasal eosinophilia

Patients with asthma demonstrate evidence of increased nasal eosinophilia, regardless of whether they exhibit clinical rhinitis (62). This correlates with the presence of bronchial eosinophilia in asthma (62).

Nasal eosinophilia has been demonstrated to be predictive of the presence of airway hyperresponsiveness in subjects with persistent perennial rhinitis suggesting that these subjects may be developing asthma pathophysiology (67).

1.3.5 Treating Allergic Rhinitis improves Asthma

In children, early recognition of AR and treatment with specific immunotherapy has been shown to decrease the severity of concomitant asthma and to reduce the risk of subsequent asthma in those without the condition (68).

There have been a number of studies that demonstrate the efficacy of the simultaneous treatment of persistent AR and asthma using intranasal corticosteroids (69-71). This suggests that treating the upper airway may have indirect anti-inflammatory benefits on the lower airways, possibly by reducing cell trafficking to the lower airways from allergic stimuli that enter the nasal passages. A recent study in children with asthma and intermittent AR demonstrated improvements in exercise-induced bronchoconstriction through the use of intranasal corticosteroids alone, without orally inhaled corticosteroids (72, 73).

The progress of both conditions tends to be linked, with improvement in one condition leading to resolution in the other and worsening in one condition resulting in persistence of the other (74-82).

1.3.6 Common pathophysiological process

Overall, the pathophysiological processes occurring in the upper and lower airways in both conditions are considered to be local manifestations of a generalized inflammatory respiratory disorder, in which eosinophillic infiltration plays a significant role (83, 84).

1.3.7 Influence of nasal obstruction upon the lower airway

An additional, independent link between upper and lower airway disease is that patients with nasal obstruction will breathe orally rather than nasally. The consequent loss of heat and humidification of inspired air may exacerbate bronchial hyperresponsiveness (85).

1.4 Immune system in AR and Asthma

1.4.1 T cells

T cells or T lymphocytes, mature in the thymus and are identified by the presence of the T cell receptor (TCR). T cells play a key role in co-ordinating adaptive immune responses to pathogens, self-antigens and environmental antigens. T cells are broadly classified into two major classes depending on whether they express the CD4 or CD8 antigen on their surface. Cells in the CD4+ "Helper" class recognise peptide fragments bound to MHC type II antigens. Upon activation, these cells secrete cytokines that promote antibody production and the activation of effector cells and mechanisms associated with host defence (86). Cells in the CD8+ "Cytotoxic" class recognise peptide fragments bound to MHC type I antigens. They function to destroy tumour cells and cells infected by viruses.

1.4.1.1 Th1 and Th2 cells

CD4+ T cells are traditionally further divided into Th1 (T helper 1) and Th2 (T helper 2) subsets. This classification scheme is based on the cytokine profile associated with each cell (87). Th1 cells are characterised by their production of interferon–γ (IFN-γ) and Tumour necrosis factor-alpha (TNF-α) and are associated with protection against intracellular bacteria, as well as autoimmune disease (88). Th2 cells produce the cytokines IL4, IL5, IL9 and IL13 (89) which promote humoral responses and antibody production and are important for defence against helminths. Th2 responses are also associated with allergic disease.

1.4.1.2 Th1 vs Th2 differentiation

Th1 and Th2 subsets are formed through the maturation of naïve Th0 cells, following their interaction with antigenic fragments on antigen presenting cells (APCs). The decision as to which subset a naïve Th0 cell will enter is determined by several factors including the allergenicity of the fragments, the

cells present nearby and the chemical microenvironment surrounding the cells (87).

1.4.1.3 CD4+ T cell – antigen interaction

CD4+ T cell interactions with antigen commence when a T cell binds to processed antigenic fragments attached to MHC class II molecules on the surface of an APC, of which DCs are the most important. Depending on the presence or absence of specific co-stimulatory molecules and the presence of specific chemical signals, one of several scenarios may arise:

- T cell maturation – Th1 pathway
- T cell maturation – Th2 pathway
- Tolerance
- Other pathways – discussed below

Th1 Pathway

Initiation of a Th1 maturation pathway appears to be prompted by the DC expressing IL-12. IL-12 also strongly suppresses the Th2 pathway. IL-12 production is prompted by immature DCs interacting with microbial fragments through Toll-like receptors (TLR) that recognize pathogen-associated molecular patterns (PAMPs) (90)

Th2 pathway

The Th2 maturation pathway is not as well understood. The trigger for the development of the Th2 maturation pathway appears to be the DC reaching full maturity during contact with an antigen. This may occur when DC-antigen contact is accompanied by an inflammatory stimulus, such as concomitant environmental lipopolysaccharide (LPS) exposure (15). IL-4 appears to play a pivotal role but the signalling and interactions between the DC and the maturing T cell are less clear. Thymic stromal lymphopoietin (TSLP) is an IL-

7-like cytokine, produced by epithelial cells, that has been suggested as playing an important role in the process (91).

Tolerance

An antigen that does not result in the activation of an immune response is said to induce tolerance. Tolerance is the usual outcome of the inhalation of harmless antigen. In order to induce tolerance, the antigen must only stimulate the production of limited numbers of effector T cells (Th1/ Th2) with increased numbers of T regulatory (Treg) cells. This typically occurs where the DC only achieves partial maturity following antigen-DC interaction (92).

1.4.1.4 Costimulatory molecules

Following binding of a CD4+ T cell to antigen fragments attached to the MHC class II molecule on the surface of an APC, for the T cell to become activated requires additional binding of co-stimulatory molecules on the APC surface (93). Such co-stimulatory molecules can influence T cell activation, maturation and function. Co-stimulatory molecules thought to play a role in T cell activation include Inducible costimulator (ICOS) and B7-related protein-1 (B7RP-1), CD30, OX40, 4-1BB, CD80, CD86 and CD28 (92).

The molecules most involved in the Th2 pathway include ICOS, CD30, OX40 and possibly 4-1BB (94, 95). ICOS in particular contributes to B cell antibody production, particularly IgE, and is essential for the creation of memory B cells (96). Despite this, ICOS has a role in the creation by T cells of the anti-inflammatory cytokine IL-10. Other important co-stimulatory molecules include CD80 and CD86 and PDL-1, which is a negative co-stimulator (14, 97).

1.4.1.5 Memory T cells

Memory T cells are a further subset of T cells. They are identified by the presence of the CD45RO isotype, whereas non-memory naïve cells express CD45RA. Memory T cells are subdivided into central and effector memory cells based on the presence of the chemokine receptor CCR7. Central memory T cells (TCM) express CCR7 and migrate to secondary lymphoid

tissues where they proliferate upon encountering antigen, giving rise to effector T cells. Effector memory T cells (TEM), lacking CCR7, migrate directly to inflamed tissues and respond rapidly upon activation (98).

1.4.1.6 Regulatory T cells (Treg)

Traditional Th1/Th2 model

Under the traditional Th1/Th2 model, it was assumed that each pathway was mutually exclusive and that a strong response in one pathway suppressed the other (87).

Traditional model and the "Hygeine hypothesis"

The finding that allergic diseases have been increasing in developed countries in recent decades led to the formulation of the "hygiene hypothesis" (99). This theory suggested that a reduction in exposure to microbial fragments in childhood, which normally led to strong Th1 responses, was resulting in increased Th2 responses (99).

However, there are several problems with this theory, including the observations that Th1-type diseases such as inflammatory bowel disease are also increasing (100, 101), a Th2 response to helminth infection appears to prevent allergy (102, 103) and that allergen-specific Th1 responses do not appear to have a significant role in suppressing allergy in asthma (104).

T regulatory cells

An alternative explanation centres around a more recently described T cell subset, the T regulatory (Treg) cell, which acts to suppress both Th1 and Th2 subsets through the secretion of inhibitory cytokines and direct cell contact. Disturbance of Treg cells, which may normally be induced by microbial fragments, results in a loss of Th1 and Th2 control resulting in inappropriate immune responses (105).

Classification of T regulatory cells

Treg cells are broadly classified into “natural” and “adaptive” categories.

Natural Tregs (CD4+ CD25+) (nTreg) are found in all healthy individuals, express the protein forkhead box P3 (FoxP3) on their cell surface and are selected in the thymus during normal immune system development. They appear to have a role in the promotion of tolerance to self-antigens and also in immune tolerance to external antigens (106). A recent study has demonstrated two distinct nTreg subtypes: ICOS+ nTregs, which express both IL-10 and transforming growth factor beta (TGF-β) and ICOS- nTregs, which express TGF-β alone (107).

Adaptive T regs, also known as “inducible”, include both Foxp3+ and Foxp3- populations (108, 109). They arise subsequent to specific antigen stimulation and inhibit T cell responses through the production of inhibitory cytokines, particularly IL-10 and TGF-β (110). Adaptive Tregs that secrete IL-10 are known as Type 1, Tr1 or IL-10-T. Those that secrete TGF-β are sometimes referred to as Th3 cells. Suppression is also achieved through cell surface molecules including cytotoxic T-lymphocyte antigen 4 (CTLA-4) (111) and programmed death-1 (PD-1) (112, 113)

Recently, several non-CD4+ T regulatory cell subsets have been identified, including CD8+ T cells, $\gamma\delta$ T cells, DCs, natural killer cells and IL-10 producing B cells (114).

Balance of Treg/ Th2 cells in health and disease

Allergen-specific inducible Treg cells are defined by their ability to produce high levels of IL-10 and TGF-β (115) in response to specific allergens. In healthy individuals Tr1 cells outnumber Th2 cells specific for common environmental allergens (115). In allergic individuals, this balance appears to be reversed in favour of allergen-specific Th2 cells (115, 116).

Disorders of Treg function

IPEX syndrome (Immune dysregulation, polyendocrinopathy and enteropathy, X-linked) is a condition where sufferers experience severe allergic disease including food allergies, eczema, eosinophilia and raised serum IgE (117). It is due to a mutation in the gene encoding for FoxP3, a key marker for natural Tregs.

Treg numbers and activity are influenced by successful therapeutic interventions

The successful use of allergen-specific immunotherapy can result in the subsequent production of allergen-specific Treg1 cells that suppress Th2 and Th1 proliferation, along with their cytokine products (115, 118).

The successful use of inhaled or systemic glucocorticoids in asthmatics is associated with increased Foxp3 and other Treg cell markers in the peripheral blood (115, 119-121). Similar results have been demonstrated in the nasal tissues of patients with AR (122).

Treg function

Tregs function to suppress allergen-induced specific T-cell activation (114), as well as DCs and allergic inflammatory effector cells including mast cells, eosinophils and basophils (123). Tregs influence immunoglobulin through the suppression of IgE production as well as class-switching to non-inflammatory IgG4 and IgA (115, 124).

Natural Tregs act largely through direct cell-to-cell contact whereas inducible Tregs act primarily through the expression of regulatory cytokines, although there is overlap in both subsets (115). Tregs interact directly with DCs to prevent DC maturation (125). They also express CTLA-4, which binds CD80/86 with a higher affinity than CD28, thus inhibiting T cell activation (126). Inducible Treg function is largely instituted through the actions of IL-10 and TGF-β (114). Natural Treg function is not always dependent on IL-10

(106). This finding may be explained by the recent discovery of the distinct ICOS- nTreg subset, which does not act through IL-10 (107).

Treg-generated IL-10 regulatory actions

IL-10 regulates Th2 responses by inhibiting the production of pro-inflammatory cytokines, and through the inhibition of APC (mostly DC), mast cell and eosinophil function along with T cell activation (Figure 1.4.1). IL-10 appears to inhibit only those T-cells that are stimulated by low numbers of triggered T cell receptors and are dependent on CD28 co-stimulation (115). IL-10 inhibits the CD28 signalling pathway by blocking CD28 tyrosine phosphorylation, preventing the binding of phosphatidylinositol 3-kinase p85 (115). Furthermore, IL-10 shifts antibody production from IgE towards non-inflammatory IgG4 (115). TGF-β plays a similar role in shifting antibody production towards IgA (115).

The role of IL-10 has been demonstrated to be critical within murine mucosal surfaces, such as the lung (127-129) and gut (130), in order to suppress allergic responses. However, IL-10 is not a pre-requisite for suppression of systemic autoimmunity in the peripheral circulation (106).

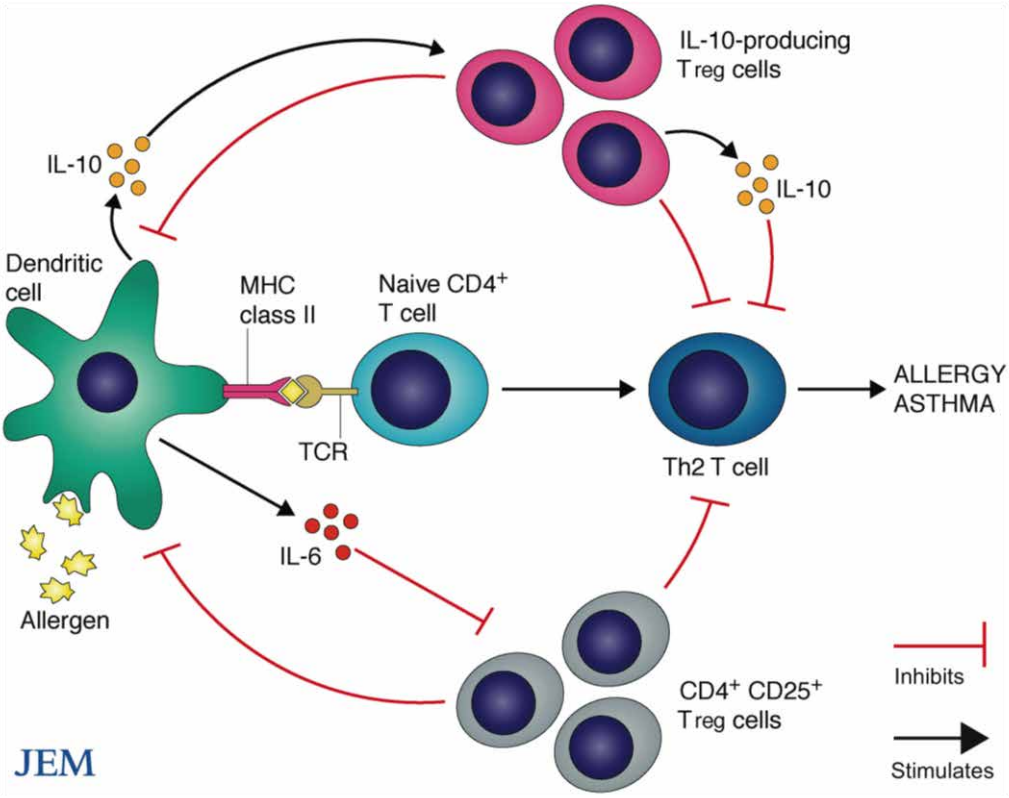


Figure 1.4.1 (From Hawrylowicz *et al*) Control of allergic airway disease by regulatory T cells. Allergic airway disease is caused by inappropriate Th2-driven immune responses to "harmless" allergens. CD4+CD25+ and IL-10–producing T reg cells can regulate allergic sensitisation *in vivo* through inhibitory effects on Th2 cells or on dendritic cells (DCs) in the respiratory mucosa. T reg cells can also induce the production of the immunosuppressive cytokine IL-10 by host T cells (not shown). In turn, DCs can produce immunomodulatory cytokines such as IL-6, which has been shown to inhibit CD4+CD25+ T reg cell function, and IL-10, which has been shown to induce IL-10–producing T reg cells (110).

IL-10 levels in health and disease

IL-10 levels are increased in health and reduce in proportion with disease severity. Treatments that improve disease symptoms, such as glucocorticoids and allergen immunotherapy are associated with increased IL-10 levels in nasal tissue (122) and in the peripheral blood (131, 132) of patients who responded to treatment (115).

Difficulties with Treg identification

The study of Tregs has been hampered by the lack of a unique marker. Foxp3+ occurs in nTregs, but only in certain inducible Tregs. Furthermore, Foxp3 is intracellular, limiting its usefulness in isolating Treg cells experimentally (133). CD25 is thought to be crucial for the regulatory subset, but in vitro suppressive activity in humans has only been demonstrated with the highest levels of CD25 (CD4+CD25high) (134). Furthermore, some Foxp3+ Tcells are CD25- (133). Other markers, such as CTLA-4 and GITR, are not specific to Tregs (135, 136). CD127 has been proposed as a marker for non-Treg T cells (133).

1.4.1.7 Th17

A subset of T cells outside the traditional Th1/ Th2 model, that produce IL-17 and are known as Th17 cells, has recently been described (137, 138). They appear to have roles in neutrophil recruitment and activation (139) along with allergy and autoimmune disease (140, 141). Animal models appear to suggest that these cells may confer resistance to corticosteroids (142). Th17 cells and their associated cytokines, IL-1, IL-6 and IL-17, have been demonstrated in neutrophil-predominant NP patients, particularly from South-east Asian populations (143).

1.4.1.8 Th17/Treg balance

There is some evidence that Th17 cells and Treg cells have reciprocal functions. The growth factor for Treg cells, IL-2, inhibits Th17 differentiation and *vice versa* for the Th17 growth factor IL-25 (144). Furthermore, the transcription factor for natural Treg cells, Foxp3, can bind and inhibit the Th17 transcription factor, retinoid orphan nuclear receptor (ROR) (145, 146)

1.4.1.9 iNKT

A class of T-cells that express an invariant T cell receptor along with specific natural killer markers are known as iNKT cells (147). These cells may be capable of rapidly producing large quantities of Th2 type cytokines (148).

Recent studies have suggested that these cells may contribute to the roles previously thought to be played exclusively by Th2 cells in asthma (149), although other studies have found otherwise (150, 151).

1.4.2 Antigen presenting cells

Antigen presenting cells (APC) process foreign antigens and present the processed peptide fragments to T cells. The processed fragments are presented together with MHC on the APC surface. T cells interact with this complex via their T cell receptor (TCR).

1.4.2.1 Professional vs non-professional APCs

Many cells can present antigen to CD8+ T cells via MHC class I. These are termed “Non-professional APCs”

Professional APCs are those that express MHC class II on their surface. Professional APCs can activate naïve T cells and stimulate CD4+ and CD8+ T cells.

1.4.2.2 Professional APC types

Professional APCs are broadly grouped into the following:

- Dendritic cells (DC) – these are the most common and most important APC
- Macrophages
- B Cells – these only present specific antigens
- Certain epithelial cells

1.4.2.3 Dendritic Cells

DCs are found principally within the skin (Langerhans cells) and mucosal surfaces. Immature DCs are also found in the circulation.

Within the respiratory mucosa, DCs are found in a network immediately above and beneath the basement membrane (152, 153). From this position, DCs form long extensions throughout the epithelium and beyond the epithelial tight junctions to the airway lumen, whilst maintaining the epithelial barrier function. Through these extensions, DCs within the nasal mucosa are able to take up inhaled antigen directly from the airway lumen without otherwise compromising the epithelial barrier integrity (154).

1.4.2.4 DC Subsets

Two major populations of DCs have been identified. The first, myeloid DCs, include interstitial DCs and Langerhans cells. The second, plasmacytoid DCs, are mainly located in the blood and secondary lymphoid organs (155). Myeloid DCs express the myeloid marker CD11b and have traditionally been thought to be derived from the bone marrow. By contrast, plasmacytoid DCs express the CD45 isoform (B220) that is normally expressed by B cells and have traditionally been thought to be of lymphoid origin. However, the concept of a distinct pathway for each DC subtype has been challenged by the recent observation that plasmacytoid DCs are able to develop into myeloid DCs under the influence of viral infections (156).

Functionally, Myeloid DCs possess potent antigen-presenting capacity and are associated with T cell activation and the initiation of adaptive immunity. By contrast, plasmacytoid DCs possess only a limited capacity to activate naïve T cells and constitute an essential component of innate immunity. This is achieved through the secretion of various cytokines and chemokines as well as by participating in the activation of natural killer cells (156, 157).

1.4.2.5 DC Function

Tissue resident respiratory tract mucosal DCs are immature. Upon antigen uptake in the presence of a danger signal, DCs undergo maturation (158).

Following antigen uptake and processing, DCs transport the antigen from the mucosa to the draining lymph nodes. In the case of the nasal mucosa, these

are the cervical lymph nodes (154), whereas in the lung, these are the mediastinal lymph nodes (158). The DCs migrate to the T cell-rich area of draining lymph nodes where naïve T lymphocytes continuously pass by (159, 160).

Upon encountering specific T cells, DCs undergo a stable interaction with these cells, leading to T cell activation, division and differentiation (161). The interaction is a three step process consisting of:

1. T cell receptor and MHCII interaction
2. Costimulation by costimulatory molecules
3. Ligation of pattern recognition receptors, such as Toll-like receptors, leading to a polarizing signal that promotes the development of either Th1, Th2 or Treg cells (162).

Different DC subsets and distinct chemical micro-environments appear to direct the development of distinct Th populations, including Th1, Th2 and Treg subgroups (158, 162).

1.4.3 Cytokines

The term cytokine refers to a group of protein cell regulators including interleukins, lymphokines, monokines and interferons, which initiate and mediate intercellular communications, controlling growth, differentiation, function and death of cells (163, 164). Most cytokines act on the local environment through autocrine (self) and paracrine (nearby) mechanisms. Some cytokines also act at distant sites in a hormone-like mechanism.

The precise source of inflammatory cytokines in AR and other eosinophilic respiratory diseases, such as nasal polyposis (NP) remains to be completely determined. *In situ* hybridisation and immunohistochemistry techniques have demonstrated that cytokines can be released by T-cells, eosinophils, mast cells, basophils and epithelial cells (165-168).

In AR, initial release is likely to be via IgE dependent mechanisms with subsequent and sustained release through the inflammatory cascade (169).

1.4.3.1 Interleukin 5

In humans, interleukin 5 (IL-5) is a 115 amino acid long Th2 cytokine. It is produced predominantly by T helper-2 cells, although it can also be produced by eosinophils (170), mast cells (22) and epithelial cells (171, 172).

IL-5 is a cytokine highly specific for eosinophils, mediating eosinophil growth and differentiation (173-176), chemotaxis and migration (175, 177, 178), activation and effector function (175, 179, 180), and survival (175, 181). Its other functions include stimulation of B cell growth and increasing immunoglobulin secretion.

Anti IL-5

Observation of the importance of IL-5 in eosinophilic-type conditions such as asthma, AR and NP has led to considerable interest in the development of anti-IL5 therapies. Initial animal experiments demonstrated significant efficacy in suppressing inflammatory cell migration and airway hyperreactivity in a monkey model of allergic asthma using reslizumab, neutralising humanised mAb against IL-5 (182). Unfortunately, similar experiments in humans, despite reducing eosinophilia in the tissues and in the blood, have failed to demonstrate clinical efficacy (183, 184). However, two recent small studies using mepolizumab have since demonstrated clinical improvement (asthma exacerbations, asthma-related quality of life measures and prednisone requirements), but only in patients with severe refractory eosinophilic asthma (185, 186).

1.4.3.2 Interleukin 10

IL-10 is a pleiotropic anti-inflammatory cytokine. It is produced particularly by Treg cells, but also by Th0, Th1, Th2 cells, B cells, DCs, macrophages, mast cells, monocytes and keratinocytes (187, 188). IL-10 is generally considered a Th2-type cytokine and potently inhibits Th1-type responses (189).

IL-10 plays a critical role in limiting and terminating inflammatory processes, particularly allergic inflammatory responses (189, 190). It acts through several

mechanisms to block pro-inflammatory cytokine production and regulate the differentiation and proliferation of several cell types including T-cells, DCs, B cells, mast cells, eosinophils and macrophages (191).

IL-10 down-regulates the expression of T cell derived cytokines including IL-2, IL-4, IL-5 and interferon gamma (IFN-γ) (192-194).

As previously discussed, IL-10 selectively inhibits only those T cells that are stimulated by low numbers of triggered T-cell receptors, which are dependent on CD28 co-stimulation (115). This is achieved through inhibition of the CD28 signalling pathway (115). T cells which receive a strong signal from the T-cell receptor alone and do not require CD28 co-stimulation are not affected by IL-10 (195).

IL-10 exerts its functions on T cells through activation of Janus Kinase (Jak 1) and tyrosine kinase (Tyk2), members of the receptor-associated Janus Tyrosine kinase family, along with signal transducer and activator of transcription 1 (Stat1), Stat3 and in certain cells, Stat5 (196). IL-10 activates src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP-1), an intracellular tyrosine phosphatase (92). SHP-1, once active, suppresses the T cell costimulatory molecules CD28 and ICOS (92). In this model, IL-10 effectively raises the threshold for T-cell activation during an APC/ T-cell interaction (92).

IL-10 plays a key role in T-cell anergy. This is essential in tolerance to allergens and autoantigens, transplantation antigens and tumour antigens (115).

IL-10 has a stimulatory effect on B cells, acting to shift antibody production away from IgE towards non-inflammatory IgG4 (115). Both total and allergen-specific IgE is reduced (118, 197).

IL-10 inhibits the expression of MHC class II, CD80 and CD86 on DCs and macrophages (189, 198). This markedly affects antigen presentation and T-cell activation.

IL-10's potent inhibition of macrophage activation results in reduced expression of pro-inflammatory cytokines (TNF-α, IL-1, IL-12, IL-6, GM-CSF), inflammatory enzymes (cyclo-oxygenase 2, inducible nitric oxide synthase) and chemokines (eotaxin, RANTES) (189).

IL-10 reduces the release of pro-inflammatory cytokines by mast cells (194) and downregulates eosinophil function, activity and survival (199, 200).

IL-10 supplements its inhibitory activity through the release of certain antagonists including soluble TNF receptors, IL-1 receptor antagonist (IL-1RA) (201, 202) and tissue inhibitor of MMP (TIMP1) (203).

The immunosuppressive role of IL-10 has been extensively studied in successful allergen-specific immunotherapy and a summary of its actions are listed in Table 1.4.1 (115).

Table 1.4.1 Mechanisms of action of IL-10 that aid the immune system as observed during allergen-specific immunotherapy (115).

IL-10
▪ Suppresses allergen-specific IgE ▪ Induces allergen-specific IgG4 ▪ Blocks B7/CD2c costimulatory pathway ▪ Inhibits DC maturation, leading to reduced MHC class II ▪ Reduces release of pro-inflammatory cytokines by mast cells

1.4.3.3 Interferon-Gamma

IFN-γ is a dimeric cytokine. It has a critical role in the Th1 pathway, promoting Th1 differentiation, whilst suppressing the Th2 pathway. IFN-γ promotes B-cell isotype switching to IgG2a and regulates MHC class I and II protein expression and antigen presentation. As well as its immunoregulatory role, it

has functions in innate and adaptive immunity against viral and intracellular bacterial infections and in control of tumours (204).

IFN-γ is produced by Th1 cells as well as Natural Killer (NK) cells, macrophages and cytotoxic T and B cells (204, 205).

1.4.3.4 Interleukin 4

IL-4 is an important Th2 cytokine. It is mainly produced by Th2 cells, mast cells, basophils and eosinophils, natural killer (NK) T cells and gamma delta T cells (206).

IL-4 regulates the protective immune response against helminths and other extracellular parasites (204). IL-4 has a key role in transforming naïve helper T cells (Th0) to Th2 cells. Conversely, it suppresses Th1 cell development. B cell proliferation is promoted via IgE class-switching, increased expression of MHC class II, up-regulation of B-cell receptors and increased expression of CD23 (204).

1.4.3.5 Interleukin 2

IL-2 is an important Th1 cytokine. IL-2 is produced mainly by CD4+ and CD8+ T cells. It is also produced by activated DCs and NK and NK T cells (207).

IL-2 has pro-inflammatory properties including the stimulation of B-cells and antibody synthesis. It promotes proliferation and differentiation of NK cells (208). However, it also has anti-inflammatory properties. It is required for Treg cell development and can suppress Th17 cells (144).

1.4.3.6 Tumour necrosis factor alpha

Tumour necrosis factor-alpha (TNF-α) is a pleiotropic inflammatory Th1 cytokine. TNF-α is mainly produced by macrophages, although it may be produced by other cells, including lymphoid cells, endothelial cells, and fibroblasts (209).

TNF-α plays an important role in the immune response against bacteria, viruses, fungi and parasites. It also has a significant role in the necrosis of specific tumours. TNF-α is able to induce local and systemic inflammation through several mechanisms. It is an acute phase protein and activates a cascade of cytokines and increases vascular permeability. It recruits macrophages and neutrophils to sites of inflammation (209).

1.4.3.7 Cytokine production during the late phase allergic response

The early phase allergic response is characterised by the release of pre-formed pro-inflammatory mediators. Such degranulation of cells occurs following the cross-linking of membrane-bound IgE. However, the late phase allergic response is characterised by the formation of pro-inflammatory cytokines and chemokines, which promote the recruitment, activation and perpetuation of cells in the inflammatory infiltrate (32). In particular, eosinophils are influenced by IL-5 along with Granulocyte-Macrophage Colony-Stimulation Factor (GM-CSF), eotaxin and Regulated on Activation Normal T Expressed and Secreted (RANTES) (210-214). mRNA levels of IL-5, IL-4, IL-10 and IL-13 (215) have been demonstrated to be increased in nasal mucosa following allergen provocation. The principal source is thought to be Th2 cells (216), with contributions from eosinophils and mast cells (21, 216, 217).

1.4.3.8 Cytokine production in chronic disease

Recent studies have demonstrated the capacity of eosinophils to produce T-cell cytokines, particularly IL-5, in significant quantities when in an activated state (218). Furthermore, immunohistochemistry has demonstrated that most IL-5 positive cells in allergic mucosa are eosinophils rather than CD3+ cells (219). This has led to the proposal that, whilst T cells are the main source of IL-5 and inflammatory cytokines initially, in the long term, the contribution of activated eosinophils is of significance for the maintenance and progression of disease (219)

1.5 Models of allergic disease

Traditionally, allergy was thought to reflect excessive Th2 cytokine production upon exposure to allergen. Non-atopics were thought to have either no significant immunological response to allergen, or a Th1 mediated response. However, recent evidence suggests a more complex interaction between Th1 and Th2 subsets, along with other, newly discovered, discrete T cell populations. The contemporary view, based on studies of allergen-specific responses by peripheral blood T cells, is that the balance of allergen-specific Th2/Th1 cells and Treg cells may, at least partly, determine a host's susceptibility to allergic disease (108, 220). A further confounding factor is the role of the recently described Th17 cells that mediate autoimmunity by affecting the balance of Th1 and Th2 cells along with role of modulatory molecules (Figure 1.5.1).

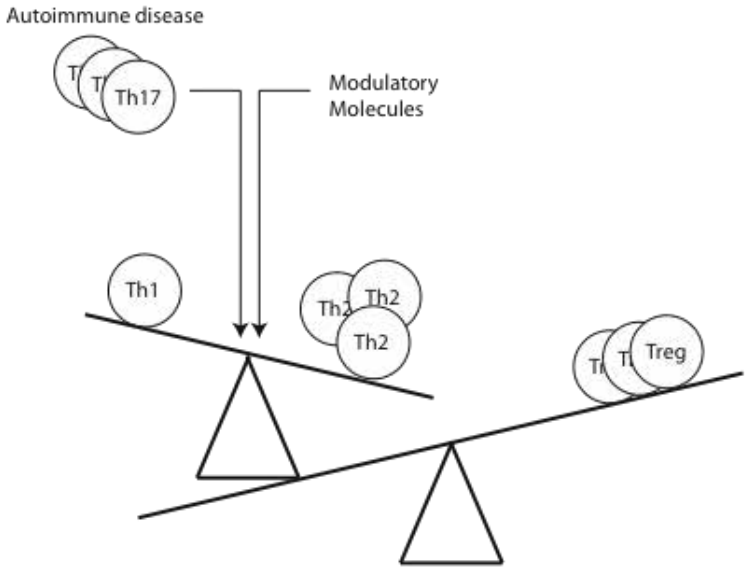


Figure 1.5.1: A host's susceptibility to allergic disease is based on a complex interaction of various cells

Animal models of allergic airway disease have proven to be very useful in expanding our understanding of disease processes. However, animal models cannot accurately represent all aspects of human disease (221).

1.5.1 Mouse models – blood and tissue

Most of the literature regarding Treg cell suppressive actions upon Th2 and Th1 pathways, to date, relates to murine experimental models. Murine peripheral blood Treg cells and, more importantly, murine respiratory tissue resident Treg cells have both been studied (128, 222-224).

In particular, murine models have facilitated testing of *in vivo* Treg cell function. Lewkowich *et al* depleted CD4+ CD25+ cells from allergy resistant immunocompetent mice prior to exposure to HDM allergen. This resulted in significantly increased Th2 responses, IgE levels, eosinophilia and airway hyperreactivity (AHR), alongside a corresponding reduction in CD4+ CD4+CD25high T cells (224).

Ostroukhova *et al* repeatedly exposed mice to low dose allergen, resulting in the formation of a regulatory T cell population. These regulatory T cells were then adoptively transferred to naïve mice, preventing allergic sensitisation (225). Similarly, Kearley et al used adoptive transfer of antigen-specific CD4+ CD25+ Tregs to suppress allergic inflammation and AHR via an IL-10 dependent mechanism (128).

While these models are useful, it is important to note that human evidence is required.

1.5.2 Human models

In humans, peripheral blood T cell responses to allergen have been studied, however, tissue evidence is lacking. The data regarding human respiratory cells is far less well established than in animal models.

1.5.2.1 Current human peripheral blood evidence

As discussed in section 1.4.1.6, human peripheral blood Treg cells, both naturally-occurring and allergen-induced, have been described (106, 131, 226, 227). Markers for peripheral blood Treg cells and their produced cytokine, IL-10, have been demonstrated to be reduced in allergic individuals

compared to non-atopic controls (115, 116, 228, 229), and increased in such individuals when successfully treated with immunotherapy and glucocorticoids (115, 118, 120, 121, 230, 231).

Children with AR sensitised to one allergen alone were followed for 2 years. Those children who remained sensitised to a single allergen had significantly higher blood IL-10 levels than those who developed sensitisation to multiple allergens (232).

Children at higher risk of developing asthma (those under 2 years of age with 3 episodes of physician diagnosed-wheeze, family history, concurrent eczema or AR) had a significantly lower number of CD4+CD25high and CD4+CD25+CTLA-4+ cells compared with healthy controls. (233)

In a study of non-atopic beekeepers, in whom bee-stings are important to maintain tolerance, it was observed that venom allergen specific CD4+ IL-10+ T cells were increased seasonally (124).

Studies of *in vitro* peripheral blood cultures demonstrated that non-atopics have significantly greater suppression of Th2/Th1 responses to allergen than atopics (106). Depletion of CD4+CD25high Treg populations from the same cultures resulted in significantly greater Th2 (IL-5) and Th1 (IFN-γ) responses to allergen in non-atopics (106).

1.5.2.2 Current human tissue evidence

Current evidence suggests that in atopic asthmatics, allergen induces IL-5 in bronchial explants (97). In AR, allergen induces IL-4 and IL-13 mRNA in *ex vivo* nasal mucosal biopsy specimens (234). There is also limited evidence to suggest that tissue-based IL-10-producing T regulatory cells are important in limiting allergic inflammation (110).

An early study demonstrated that intranasal instillation of IL-10 at the time of allergen challenge inhibited leukocyte recruitment (235).

John *et al* demonstrated a marked reduction in IL-10 mRNA and increased pro-inflammatory cytokines in bronchoalveolar lavage fluid and alveolar macrophages of asthmatic subjects compared to healthy controls (236). They subsequently demonstrated increased IL-10 mRNA and reduced pro-inflammatory cytokines in alveolar macrophages following inhaled corticosteroid use.

A study of paediatric lung tissue demonstrated reduced CD4+CD25high T cell numbers and activity along with Foxp3 mRNA in asthmatics compared to controls with cough but not asthma (237). Inhaled corticosteroids restored the studied T cell population numbers and function.

Foxp3+ cell populations and mRNA, along with CD4+CD25+ T cell numbers were demonstrated to be reduced in the nasal tissues of subjects with AR compared to controls (238). By contrast, Foxp3+CD25+CD3+ cells were increased in the nasal mucosa of AR patients following clinically successful grass pollen immunotherapy (239).

1.5.2.3 Tissue evidence restrictions

Human respiratory tissue evidence is limited partly due to the difficulty in obtaining respiratory tissue. Traditionally, in asthma, this has required bronchial biopsy, an invasive medical procedure for which it is difficult to recruit volunteers. Bronchoalveolar lavage is an indirect alternative, but a poor substitute. As a consequence, an alternative source of human respiratory tissue that can provide the necessary data, without the potential morbidity and limited availability of bronchial tissue, is required.

1.5.2.4 Alternative tissue – Nasal Polyps

Human nasal polyps provide a ready source of large numbers of respiratory cells that may be harvested incidentally during therapeutic surgical procedures.

It is well-established that allergen exposure does not induce symptoms in patients with nasal polyposis (240). Immune cells in nasal polyps have a

relatively innocuous response to allegens (240), suggesting constraint by local control mechanisms of allergen-specific Th2 responses. Nasal polyp cells are therefore a useful resource for investigating the allergic response in human airways.

1.6 Nasal Polyposis

1.6.1 Definition

Nasal polyposis (NP) is a chronic inflammatory condition of the paranasal sinus mucosa, resulting in pendulous, oedematous, epithelial lined structures that expand into the nasal cavity and sinuses (241). NP is characterised by nasal obstruction and hyposmia or anosmia (241).

1.6.2 Epidemiology

The prevalence of NP is reported to be between 1% to 4% of the adult population, with most cases occurring after the age of 20 years and peak incidence occurring between the ages of 50 and 60 (242).

Childhood cases are limited mostly to patients with cystic fibrosis (243). Nasal polyps occur in all races and social classes (244). Some authors report an increased incidence in males (2-4:1) (244), but this has not been found by others (245). Hereditary factors have been considered (244).

1.6.2.1 Risk factors

The prevalence of NP is increased in asthmatic patients at 7 – 14% (241, 245). The incidence is even higher in patients with nonallergic, steroid-dependent asthma compared to those with allergic asthma (245). Furthermore, 20-50% of patients with nasal polyps have asthma (246, 247).

Patients with aspirin sensitivity have a 60% incidence of NP (246). Around 8-36% of polyp patients have aspirin sensitivity (245, 248-250). Samter's triad which consists of asthma, aspirin sensitivity and nasal polyps is reported to occur in up to a third of NP patients (246, 247).

Other conditions associated with nasal polyps include Cystic fibrosis (243), allergic fungal sinusitis, Churg-Strauss syndrome (245), non-allergic rhinitis – eosinophilia syndrome (NARES) (251, 252), Young's syndrome (247) and primary ciliary dyskinesia (253, 254).

1.6.2.2 Nasal polyps are NOT associated with atopy

The presence of nasal polyps is not an indicator of atopy or AR (255-258), despite the association with IgE, eosinophilia, IL-5 and mast cells. Indeed, anti-histamines are not effective in treating patients with nasal polyps (259).

1.6.2.3 Subtypes

NP may be subdivided into eosionophilic-predominant NP with a Th2 cytokine profile, which form the majority of cases, and neutrophil-predominant NP with a Th1 or Th17 cytokine profile (143).

Neutrophil-predominance, rather than eosinophilia occurs in around 7% of NP cases. This is found in cases associated with CF, primary ciliary dyskinesia or Young's syndrome, all of which are steroid-insensitive (245-247, 260).

Recent reports from South-east Asian research groups have indicated that in Korean and Chinese populations, in contrast to Western populations, the majority of NP patients have a neutrophil-predominant form of the disease (261, 262).

1.6.2.4 CRSwNP and CRSsNP

NP is considered a subset of the overarching condition, chronic rhinosinusitis (CRS). CRS is divided into chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyposis (CRSsNP). Apart from the obvious differences regarding the presence or absence of polyps, these conditions may be differentiated by the expression of inflammatory and remodelling mediators. CRSwNP demonstrates a Th2-type eosinophilic inflammation with increased IL-5 and IgE and low Transforming Growth factor

B1 (TGF-β1). By contrast, CRSsNP demonstrates a predominantly Th1 environment with high IFN-γ and TGF-β1 levels (263).

1.6.3 Histopathology

1.6.3.1 Macroscopic

Macroscopically, nasal polyps are usually bilateral, multiple and movable. They appear semi-translucent, pale-gray with a smooth, glistening surface. They arise from a pedicle attached around the ostiomeatal complex, mostly from the uncinate process and the middle turbinate (260). Symptoms are primarily due to physical obstruction, with patients complaining of nasal blockage and hyposmia or anosmia. Other symptoms include headache, facial pain, post-nasal drip and rhinorrhoea (253).

1.6.3.2 Microscopic

Nasal polyps are characterised by marked stromal oedema, pseudocysts in deeper layers (264) and inflammatory cells. In particular, marked eosinophilia is found in most, but not all, nasal polyps (265-267). Non-eosinophilic polyps tend to be neutrophil predominant. Mast cells are also found in many nasal polyps, often degranulated (268-270). Other findings include proliferation of connective tissue and epithelial cells (271), basal membrane thickening (272), fibrosis (273), and deposition of fibronectin and albumin (264, 274, 275).

1.6.3.3 “Tertiary” lymphoid tissue within some nasal polyps

In some NP patients, lymphoid tissue, classified as “Tertiary”, including follicular structures and lymphoid accumulations, has been demonstrated (276-278). Such tissue has been shown to have the capacity for local IgE production, both polyclonal and specific to *Staphylococcus Aureus* enterotoxins (SAE) (276).

1.6.3.4 Differences between polyps and regular nasal mucosa

Polyps exhibit certain differences from regular nasal mucosa. The epithelium demonstrates a reduced number of ciliated cells and an increased number of goblet cells (279). Areas of squamous metaplasia or basal hyperplasia may also be seen (280). There are few nerve endings (243) and vascularisation is limited and lacking in vasoconstrictory innervation (281). There is marked stromal oedema, psuedocysts in deeper layers (264) and inflammatory cells, particularly eosinophils and mast cells.

1.6.4 Pathophysiology/ Aetiology

The aetiology of NP is unknown but believed to be multifactorial. The polyp stroma contains numerous inflammatory cells, including eosinophils, mast cells and T lymphocytes (282-284). In addition, numerous inflammatory mediators including cytokines, chemokines, growth factors, adhesion molecules, IgE and other proteins are present. The role and relative importance of each of these is yet to be fully determined (285).

Allergy does not appear to play a causative role in nasal polyps (286-288). However, the presence of high concentrations of IgE, IL-5, eosinophils and mast cells does suggest a Th2 pathway is involved in the pathogenesis, although the role of IgE remains controversial (289). Recently, it has been proposed that in NP patients that are atopic vs non-atopics, Th17 and its cytokine IL-17 may play a significant role (262). Many of these disparate findings are reflective of the numerous phenotypes and co-morbidities that exist within the definition of the condition, nasal polyposis.

Animal experiments have been hampered by the fact that the chimpanzee is the only animal known to suffer from a similar condition (248).

1.6.4.1 Eosinophilia

Marked eosinophilia is seen in 80-90% of polyp patients in Western populations (143). Asthma, atopy, and aspirin intolerance are all associated with increased eosinophilia (240, 285). In all these instances, the eosinophil is

believed to be the key cell in the inflammatory process. Eosinophil activation is associated with numerous mechanisms thought to be involved in the pathogenesis of nasal polyps including injury of epithelium, thickening of the epithelial basement membrane, stromal fibrosis, angiogenesis, and epithelial and glandular hyperplasia (265, 266, 273, 290). Many of these mechanisms are initiated and modulated through the release of cytokines, chemokines and growth factors, some of which originate from the eosinophil itself (284), including IL-4, IL-5 (283, 291), GM-CSF (282, 292, 293) and TGF-ß1 (283, 294), along with pro-inflammatory mediators including cys-LT, Eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), major basic protein (MBP) and Leukotriene C4 (LTC4) (19).

Eosinophils arise from pluripotent stem cells within the bone marrow via the actions of IL3, IL5 and GM-CSF (295). Eosinophils are recruited to nasal polyp stroma and activated primarily by the actions of IL5 in atopic patients (296). Current opinion suggests that, in the absence of allergy, GM-CSF appears to be the main factor in eosinophil recruitment (240, 296). Once within respiratory tissue, eosinophil apoptosis is delayed by the actions of IL-5 and GM-CSF (297).

1.6.4.2 Cytokines

IL-5

Several cytokines are implicated in polyp pathogenesis. However, IL-5 has consistently been found in numerous reports to be significantly elevated within tissue stroma (263, 298, 299). The eosinophil is the only human leukocyte to express a specific receptor for IL-5 (300, 301). IL-5 has been demonstrated to be essential for the recruitment, migration, activation, degranulation and survival of eosinophils in the tissue (219, 299, 302-304). The primary source of most IL-5 production is generally thought to be Th2 cells (281). However, eosinophil activation can result in increased IL-5 production from eosinophils themselves, leading to autocrine stimulation and attraction of other inflammatory cells (305). With immunohistochemistry, the majority of IL-5

producing cells seen in polyp tissue are thought to be eosinophils (219). Mast cells are another suggested source of IL-5 (306).

Other Cytokines

Granulocyte-macrophage Colony-Stimulation Factor (GM-CSF) is also over-expressed in nasal polyps and has a similar role to IL-5 in eosinophil chemotaxis (307) and survival (298, 308).

Other cytokines implicated in nasal polyp pathogenesis include IL-1 (301), IL-3 (309), IL4 (299, 301), IL6 and IL8 (284, 301), along with IFN-γ (310, 311). However, several contradictory studies have cast doubt on the significance of these cytokines (298, 299).

1.6.4.3 Chemokines

Other molecules that activate and recruit eosinophils are the chemokines eotaxin and RANTES (241, 307). Some authors have speculated that in patients with nasal polyps and allergic fungal sinusitis (AFS) or eosinophilic mucin rhinosinusits (EMCRS), eotaxin plays a greater role than IL-5 in eosinophil accumulation (312). The concentration of RANTES in nasal polyps has been subject to contradictory findings, ranging from unchanged relative to controls (298) to raised (313, 314).

1.6.4.4 Growth factors

Tissue remodelling and structural modifications in nasal polyps require the presence of growth factors and matrix metalloproteinases (MMPs). Transforming Growth factor B1 (TGF-β1) has been linked in nasal polyps with chemotaxis and activation of fibroblasts, increased production of proteins in the extracellular matrix, and inhibition of the production of enzymes that degrade the extracellular matrix components such as collagenase, heparinase and stromelysin (315-317). TGF-β1 has anti-inflammatory activity, decreasing IgE synthesis and eosinophil activation (318). Within polyps, it appears to be localized to the zones of extracellular matrix accumulation and stromal-cell

(myofibroblasts) infiltration (283). TGF-β1 is increased in nasal polyps compared to normal mucosa (294).

Other growth factors that may play a role include Platelet Derived Growth Factor (PDGF) and Vascular Endothelial Growth Factor (VEGF) (294, 319).

The adhesion molecules E-Selectin, P-Selectin and vascular cell adhesion molecule 1 (VCAM-1) also appear to be increased in nasal polyps (320-322).

1.6.4.5 IgE

Increased levels of IgE have been demonstrated in polyp patients, particularly those with atopy (245, 323, 324). In some cases, this IgE has been demonstrated to be locally produced within NP tissue (276).

The high levels of IgE seen in atopic and non-atopic polyp patients suggests the IgE-dependent degranulation of effector cells, such as mast cells. The receptors that bind IgE to mast cells, FcεRI and FcεRII (CD23) have been observed on eosinophils also (325, 326). The IgE seen in non-atopic polyps is not specific for the major allergens seen in atopy (288). This suggests that pathogenisis may require an IgE dependent but non-specific degranulation of effector cells including mast cells and eosinophils (288). This theory may unify the observed high concentrations of IgE, IL-5 and eosinophils (288).

1.6.4.6 Theories of causation

The exact aetiology of NP remains unknown. Several theories have been proposed as possible explanations.

Staphylococcus superantigen

Recent studies have demonstrated increased IgE levels against *staphylococcus aureus* enterotoxins (SAE) within polyp tissue (258). Individual *S aureus* products induce various effects on nasal polyp mucosa. Surface protein A (SpA) induces mast cell degranulation, whereas enterotoxins induce Th2 type cytokines including IL-5, eotaxin, IL-2 soluble

receptor α, and IgE (263). Furthermore, in NP, increased rates of *S aureus* colonisation have been demonstrated (327). These findings support the possible role of *staphylococcus* superantigens in the development of nasal polyps.

Injury and epithelial wound healing

Some authors have proposed a model of polyp formation based on epithelial injury and dysregulated wound healing (328). Evidence is derived from animal experiments involving rat middle ears and rabbit maxillary sinuses (328-330). Epithelial injury was created and bacteria introduced to stimulate an inflammatory reaction. Connective tissue containing fibroblasts and inflammatory cells herniated through the epithelial lesion and was subsequently re-epithelialised. The resultant polyp-like structures closely resembled human nasal polyps with oedema and marked inflammatory cell infiltrate (328-330).

Polyps typically arise between narrow areas of mucosal contact, such as the middle meatus. Chronic irritation in these narrow areas may provide the initial stimulus for polyp formation (259). The narrow areas may also provide the ideal environment for *staphylococcus* superantigens to persist (331).

Vasomotor-imbalance theory

Other authors suggest polyp formation is based on limited vascularity and poor vasoconstrictory innervation, resulting in reduced venous drainage and impaired clearance of degranulation products such as histamine. Prolonged histamine action results in increased stromal oedema (246-248, 332). In support of this concept is the finding that histamine concentrations in polyps are 100-1000 times higher than in serum (247, 248).

1.6.4.7 Aspirin sensitivity

A subgroup of NP patients are sensitive to aspirin and non-steroidal anti-inflammatories (NSAIDS). Aspirin sensitive patients experience abnormal regulation of cyclo-oxygenase pathways. The anti-inflammatory prostaglandin

E2 (PGE2) is reduced in these patients compared to normal controls and aspirin-tolerant polyp patients (333). Decreased PGE2 production leads to enhanced 5-lipoxygenase activity and overproduction of cysteinyl leukotrienes, which result in mucosal inflammation (334).

1.6.5 Clinical Features

The hallmarks of NP are nasal obstruction and impaired olfaction. Other symptoms are similar to those observed with CRS, including rhinorrhoea, post-nasal drip and facial pain (241).

1.6.6 Management

1.6.6.1 Topical steroids

Topical glucocorticords can be useful in the treatment of nasal polyps (241). The clinical efficacy of topical steroids is thought to be a result of the reduction in eosinophil infiltration (241). Neutrophil-predominant polyps, such as those in patients with cystic fibrosis or primary ciliary dyskinesia, are less likely to respond to topical steroids [10].

1.6.6.2 Systemic Steroids

Oral corticosteroids are highly effective in the treatment of nasal polyps (241). Symptoms of obstruction and reduced olfaction respond almost immediately to the commencement of medication. Systemic steroids exert their effect on nasal polyps through various mechanisms, both genomic and non-genomic (335). However, the primary mechanism is thought to be due to a dehydrating effect on the polyps, which typically have a high fluid content (241).

The usefulness of oral steroids is limited by systemic side effects. Typically, a brief course of oral therapy (20 days or less) will provide relief for up to 8 weeks (336).

1.6.6.3 Surgery

Surgical removal of nasal polyps provides rapid relief of symptoms with the incidental benefit of an abundant source of tissue for laboratory investigation. Unfortunately, recurrence is common, with the time to revision surgery varying between months to decades later.

Recurrence following surgery is more likely in patients with asthma, with positive skin prick tests and with aspirin intolerance (243, 248).

Medical management may be used to delay or avoid surgery. The mainstay of medical treatment remains topical or systemic steroids (248, 337-339).

1.6.6.4 Adjuvant therapies

Anti IL-5

There is considerable interest in the use of anti-IL5 therapy in the treatment of NP. To date, however, there has been little evidence of clinical efficacy. A small (n=24) safety and pharmacokinetic trial demonstrated an improvement in NP symptom scores in half the patients treated with a single intravenous infusion of reslizumab vs placebo (340).

Monteleukast

In patients with Samter's triad (NP, asthma and aspirin-sensitivity, see section 1.6.2.1), there is evidence to suggest that the cysteinyl leukotriene receptor antagonist, montelukast, is effective in reducing nasal symptom scores and eosinophil counts in nasal smears and peripheral blood (341).

Doxycycline

Recent studies have demonstrated that certain antibiotics, such as doxycycline, may significantly reduce nasal polyp size for up to 12 weeks, following a 20 day course (336).

2 AIMS

The aim of this thesis was to attempt to address the shortfall in evidence regarding the role of IL-10 producing regulatory T cells in suppressing allergen-specific Th2/Th1 responses in human respiratory tissue, through the use of *ex vivo* cell suspensions derived from nasal polyps.

2.1 Hypotheses

We hypothesised that:

1. IL-10 production plays a critical role in limiting inflammatory allergen-specific Th2 and/or Th1 responses following exposure of *ex-vivo* human respiratory cells, derived from nasal polyps, to allergen.
2. Allergen-specific, tissue-resident T cells play a key role in the production of this IL-10.
3. These tissue-resident T cells represent a specific population of regulatory T cells that act to suppress abnormal immune responses to allergen exposure and that this function is exerted through the anti-inflammatory actions of IL-10, produced by such regulatory T cells when exposed to specific allergen.

3 METHODS

3.1 Ethical Approval

The research was undertaken with the approval of The Guy's Hospital ethics committee/ King's College, London and the UK COREC (Central Office for Research Ethics Committees) (Approval Number 01/09/12). Full written informed consent was obtained from all participants prior to their involvement in the research. All consent and information forms were approved prior to use (Approval Number 01/09/12) (see Appendix 1 – Patient Information and Consent Forms)

3.2 Patient population

Non-smoking adult subjects (n=30) were recruited from the Rhinology outpatient clinic of the Otolaryngology Department, Guy's Hospital, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom. To be included in the study, subjects had to be diagnosed with NP of sufficient severity to require surgical clearance. The diagnosis of NP was made in accordance with the guidelines of the European Position Paper on Rhinosinusitis and Nasal Polyps (342), and was based upon symptoms, clinical examination, nasal endoscopy, and sinus computed tomography scan. Subjects requiring primary (n=21) or revision (n=9) surgery were included in the study as were subjects with aspirin-sensitivity (n=1).

Subjects were excluded if they had: evidence of active infection within the nose at the time of surgery; had taken oral or topical (intranasally administered) corticosteroids, antihistamines, antileukotrienes, antibiotics or nasal decongestant medication in the 2 weeks immediately prior to surgery or had received immunotherapy within the last five years.

Subject demographics are shown in Table 3.2.1.

Table 3.2.1: Subject Demographics

Gender	
Male	20 (67%)
Female	10 (33%)
Age (years)	
Mean	49
Range	29 to 78

3.3 Measurement and classification of atopic status

Prior to surgery, all subjects underwent skin prick tests to a panel of six common aeroallergens including cat, grass, house dust mite (HDM), dog, tree (silver birch) and *Aspergillus* (Soluprick, ALK, Horsholm, Denmark) alongside a positive histamine control and a negative diluent control. A positive skin prick test result was defined as a wheal response (diameter >= 3mm) to one or more allergens at 15 minutes, plus a positive histamine response and a negative diluent response. A negative skin prick test result was defined as no response or a response with a diameter < 3mm to all of the tested allergens at 15 minutes, plus a positive histamine response and a negative diluent response.

3.3.1 "Atopic" status by skin prick test alone for this study

For the purposes of this study, "atopic" status was determined by skin prick test alone, as a specific *in vivo* measure of tissue-based immunological responsiveness. Subjects were classified as *atopic* (those with a positive skin prick test response, n=20) or *non-atopic* (those with negative skin prick test response, n=10)(Table 3.3.1).

Clinical history of AR or asthma was recorded but not used to determine atopic status for the purposes of this study. *In vitro* serum IgE testing was not used to determine atopic status for the purposes of this study.

Table 3.3.1: Atopic status of recruited subjects

Subject Status	n
Non-atopic	10
Atopic	20
Cat	6
Grass	15
House Dust Mite	9
Dog	2
Tree (Silver Birch)	4
Aspergillus	0
1 positive allergen alone	9
2 positive allergens	7
3 positive allergens	3
4 positive allergens	1

No subject responded to tree or dog allergen alone, that is, those subjects who responded to tree or dog allergen also responded to at least one other allergen.

3.4 Obtaining nasal polyp tissue

Surgery was performed at a single institution (Guy's Hospital, London, United Kingdom) by one of several surgeons, including the author. Surgery was performed under general anaesthesia with either endotracheal intubation or laryngeal mask. After the induction of anaesthesia, vasoconstriction and local

anaesthesia was obtained with the topical administration of 10mls of “Moffet’s” solution (Cocaine hydrochloride 1mL of 10%, adrenaline 1mL of 1:1000, Sodium bicarbonate 2mL of 8.4%, saline 6mL) within the nose. The routine administration of intravenous steroids and/ or antibiotics was delayed until the polyps had been harvested. Polyp tissue was obtained bilaterally using Blakesley-Weil forceps. Freshly obtained tissues were placed in a container of normal saline and transported to the laboratory for processing. Specimens were kept refrigerated at 4°C until they were processed. All processing was performed within several hours of harvesting and no more than 19 hours after collection.

3.5 Isolation of cells

Tissue fragments were rinsed with saline to remove residual blood. Tissue was dissected, teased apart, and digested for one hour at 37°C using a solution of Hank’s Buffered Saline Solution (HBSS) (Life Sciences, Abingdon, UK), 2% Foetal calf serum (FCS) (PAA Laboratories, Pasching, Austria), and endotoxin-free collagenase (2 mg/mL, Liberase C1; Roche Diagnostics, Lewes, UK). The digest was then centrifuged at 200g, then re-suspended and cultured for 4 hours in a solution containing RPMI (Roswell Park Memorial Institute) 1640 medium (Life Sciences, Abingdon, UK), 10% FCS, 2 mmol/L L-glutamine (Gibco/ Invitrogen, Paisley, UK) and gentamicin (250 mg/mL) (Sigma-Aldrich, Ayrshire, UK). The digested fragments were then filtered through a 100-micron cell strainer (BD Biosciences, Bedford, MA, USA) and a single cell suspension obtained.

3.6 Depletion of T cells

For one series of experiments, T cells were selectively depleted on the basis of CD3 expression on a “MACS” magnetic column according to the manufacturer’s instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). The flow through this column typically resulted in > 95% depletion of CD3+ T cells as determined by Flow cytometric analysis (Figure 3.6.1). As a control, cells were passed through the column without the initial incubation with antibody coated magnetic beads.

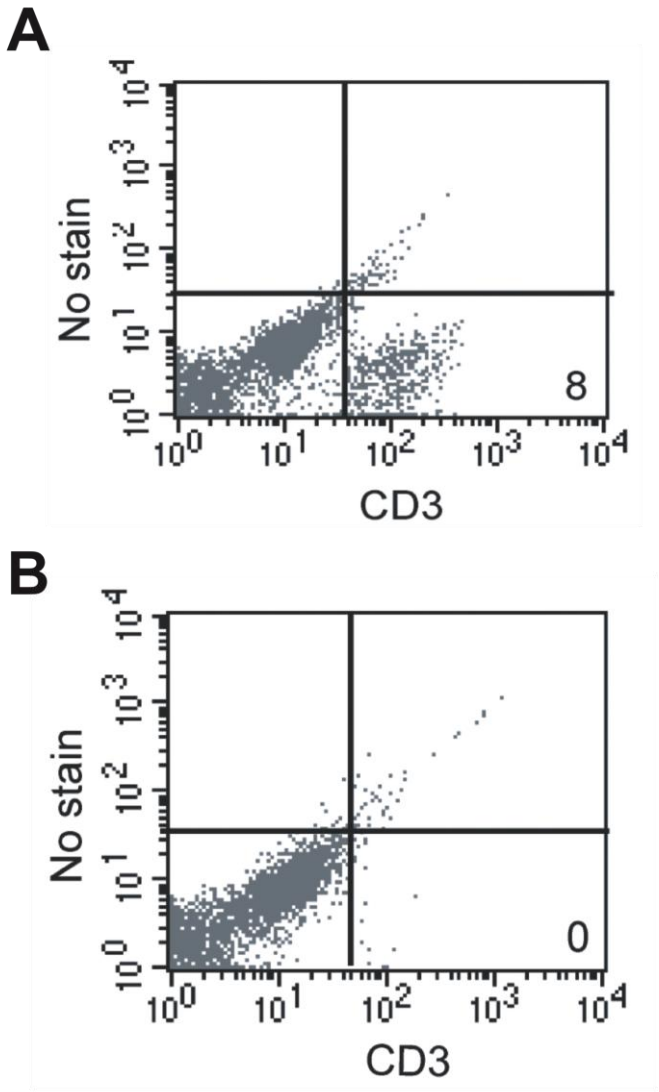


Figure 3.6.1 FACS analysis of flow through the magnetic column. (A) represents column control, (B) represents flow following CD3+ T cell depletion, demonstrating >95% depletion

3.7 Cytokine Production

Cell suspensions were seeded at 1 X 10^5 cells per well (96-well flat bottom, Nunc, Roskilde, Denmark) in complete medium (100 µL). Cell suspensions were stimulated with cat, grass or *Der p1* house dust mite allergen (Aquagen SQ, ALK, Horsholm, Denmark) at concentrations of 10^1, 10^2, 10^3 and 10^4 U/mL. 10^4 U/mL is approximately equivalent to 0.75 µg/mL protein. Initial experiments demonstrated a predictable dose response curve (Figure 3.7.1) and all subsequent experiments were carried out with allergen concentrations of 10^2 and 10^4 only.

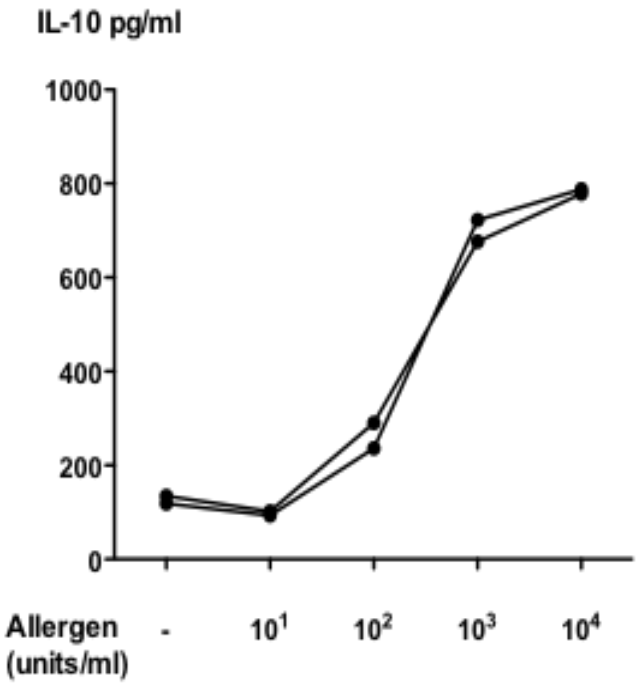


Figure 3.7.1: Interleukin 10 production at 6 days following stimulation with various concentrations of cat allergen (in HBSS diluent) in a subject allergic to cat. Note: two wells were tested for each allergen concentration. A representative experiment of 3 is shown.

For all experiments, 2 control wells were seeded to reduce the likelihood of errors.

3.7.1 Reconstitution of allergen

The dry allergen is normally reconstituted with the manufacturer's diluent, which contains phenol as a preservative. During initial experiments, the manufacturer's diluent was compared with HBSS as a diluent. These experiments demonstrated that, at higher concentrations, the manufacturer's diluent attenuates the cytokine response compared to HBSS (Figure 3.7.2), presumably due to the actions of phenol. In all subsequent experiments, HBSS was used as the reconstituting agent, rather than the manufacturer's (ALK) diluent.

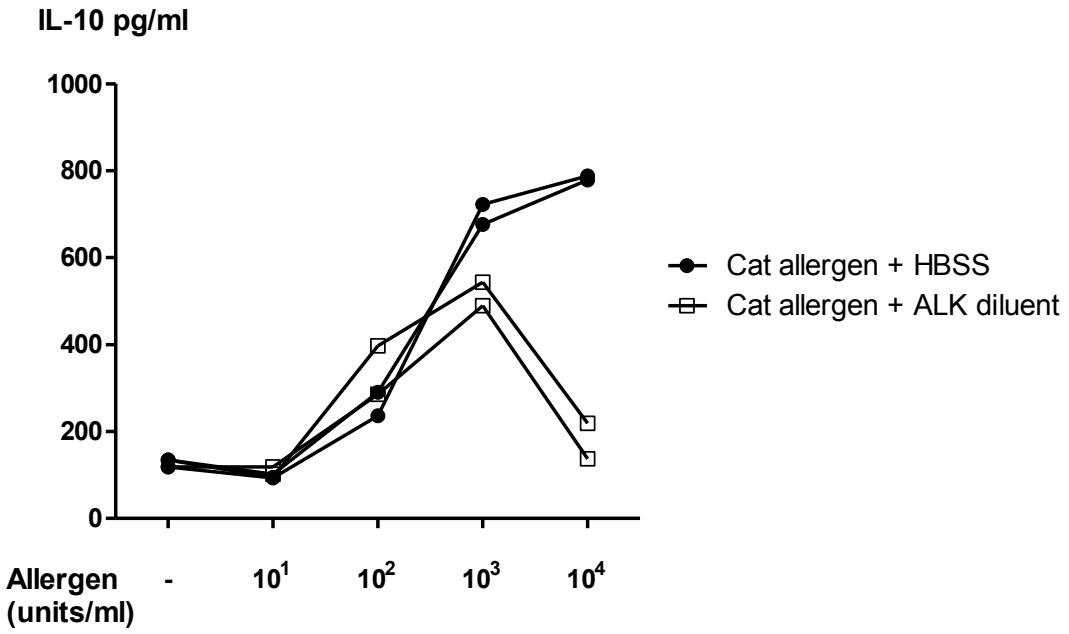


Figure 3.7.2: Interleukin 10 production at 6 days following stimulation with various concentrations of cat allergen using HBSS diluent (closed circles) or the manufacturer's (ALK) diluent (open squares) in a subject allergic to cat. Note: two wells were tested for each allergen concentration. A representative experiment of 3 is shown.

3.7.2 Added allergen and sensitisation

Allergen was added to cell suspensions from atopic and non-atopic individuals.

When adding allergen to cell suspensions, three possible scenarios could arise:

1) The individual was non-atopic to all allergens OR
2) The individual was atopic and was sensitised to the added allergen OR
3) The individual was atopic and was not-sensitised to the added allergen (ie sensitised to a different allergen to that added)

For example, when adding cat allergen to a cell suspension, the individual from whom the cells were derived could be:

1) Non-atopic to all allergens OR

2) Atopic to cat ("sensitised" allergen added) OR

3) Atopic to grass, but not cat ("non-sensitised" allergen added)

3.7.3 6 Days culture

Peripheral blood mononuclear cell cytokine production is optimal at around 6-days (97). We performed time course experiments comparing day 6 with day 2 (data not shown) and there was no significant difference in cytokine levels. Accordingly, 6 days culture was used for all subsequent experiments.

3.7.4 Cell viability at 6 days culture

Cell viability was maintained for the period of culture in representative experiments at 77± 9% (mean ± SEM).

3.7.5 Antibodies

Monoclonal antibodies were obtained:

anti-IL-10 (BD Pharmingen, Cowley, Oxon, UK)

Control antibodies (Rat isotype, mouse isotype) (BD Pharmingen, Cowley, Oxon, UK)

A panel of fluorochrome conjugated monoclonal antibodies to leukocyte surface markers was obtained:

CD45, CD326, CD14, CD16, CD117, CD3, CD19, CD4, CD8, and CD127 (BD Pharmingen, Cowley, Oxon, UK), CD103 (eBioscience, Hatfield, UK)

mAbs to CD3 were prepared in-house

mAbs to CD28 (Sanguin, Amsterdam, Netherlands)

3.7.6 Supernatants harvested

Following 6 days of culture, at 37°C, the cell suspension supernatants were harvested and cytokine levels measured.

3.7.7 Cytokine measurement by cytometric bead array

The cytokines IL-2, IL-4, IL-5, IL-10, TNF-α and IFN-γ were measured simultaneously by cytometric bead array using the Human Th1/Th2 kit from BD Biosciences, Bedford, MA, USA, according to the manufacturer's instructions. The lower detection limit of the assay for each cytokine was 5 pg/mL.

3.7.8 Evidence of T-cell presence and activity by polyclonal stimulation

In a representative set of experiments, polyp cell suspensions were stimulated with anti-CD3/CD28 mAb, a polyclonal T-cell activator to confirm T cell presence and potential activity (Table 4.5.2 and Figure 5.5.7)

3.8 Statistical Methodology

Data were analysed using GraphPad Prism 5 (GraphPad Software Inc, San Diego, CA, USA). Differences between paired groups with non-parametric data were tested with the Wilcoxon Signed Rank test. Differences between unpaired groups with non-parametric data were tested with the Mann-Whitney test. All tests were performed two-sided. A p value <0.05 was considered statistically significant.

3.8.1 Analysed population

Of the 30 subjects recruited into the study: two subjects' cultures were excluded as the allergens were diluted solely with the manufacturer's diluent (containing phenol); one was excluded because the cell suspension became infected during incubation; two sets of experiments were excluded as the cytokine production was assessed at day 2 alone and not day 6. Results from the remaining 25 experiments were included.

In five of the remaining experiments, there was no significant production of IL-5 or IL-10 (defined as all results less than 16 pg/mL): three of these cases were atopic and two were non-atopic. These cases were defined as *non-*

responders, the remaining 20 cases were defined as *responders*. *Non-responders* were excluded from the analysis, but all data are included in Appendix 2 – raw data.

3.8.1.1 Demographics of analysed population

Demographics of the 20 analysed subjects are shown in Table 3.8.1

Table 3.8.1: Subject Demographics

Gender	
Male	12 (67%)
Female	8 (33%)
Age (years)	
Mean	46
Range	29 to 71

3.8.1.2 Atopic status of analysed population

The atopic status of the 20 analysed subjects is shown in Table 3.8.2

Table 3.8.2: Atopic status of subjects analysed

Subject Status	n
Non-atopic	8
Atopic	12
Cat	6
Grass	7
House Dust Mite	7
Dog	2
Tree (Silver Birch)	3
Aspergillus	0
1 positive allergen alone	4
2 positive allergens	4
3 positive allergens	3
4 positive allergens	1

No subject responded to tree or dog allergen alone, that is, those subjects who responded to tree or dog allergen also responded to at least one other allergen.

4 CONSTITUTIVE CYTOKINE PRODUCTION

4.1 Introduction

Nasal polyp cell suspensions have been demonstrated to constitutively produce IL-5, IFN-γ and IL-10 (343-345), using Enzyme-linked immunosorbent assay (ELISA) on T cell suspensions (343), ELISA for intracellular cytokine detection (344) and reverse transcription polymerase chain reaction (RT-PCR) (345).

4.2 Aims

The aims of this experiment were:

To determine the constitutive Th1/Th2 cytokine expression from cell suspensions derived from human nasal polyps.

To determine the Th1/Th2 cytokine response from cell suspensions derived from human nasal polyps following polyclonal T cell activation using anti-CD3/ CD28 monoclonal antibodies, as a positive control, to test the viability of the model.

4.3 Hypotheses

We hypothesised that:

Cell suspensions derived from human nasal polyps would respond with the production of IL-10, IL-5 and IFN-γ.

The addition of anti-CD3/CD28 monoclonal antibodies would result in a marked increase of all Th1/Th2 cytokines measured.

4.4 Methods

The methods used are detailed in Chapter 3.

Briefly, nasal polyps were harvested, processed, digested with collagenase and filtered to produce a single cell suspension that was seeded at 1 X 10^5

cells per well. Wells were incubated for 6 days and the cell supernatants harvested. In some cases cells were also stimulated with plate bound anti-CD3 (1 µg/ml) and anti-CD28 (1ug/ml) mAbs and supernatants harvested after 48 hrs of culture. CBA analysis was performed to determine cytokine levels.

4.5 Results

4.5.1 Constitutive cytokine production

Cell suspensions derived from human nasal polyps responded with the production of IL-10, IL-5 and IFN-γ. Constitutive production of IL-2, IL-4 and TNF-α was negligible (Table 4.5.1).

The combined results for all subjects (12 atopic and 8 non-atopic, 20 total) are presented.

Table 4.5.1: Constitutive cytokine production. Cell suspensions derived from human nasal polyps were cultured for 6 days and cytokine production was assessed by CBA. (n=20) showing mean ± SEM in pg/mL

Cytokine	Mean ± SEM (pg/mL)
IL-10	62±11
IL-5	93±21
IFN-γ	191±54
IL-2	16±2
IL-4	7±1
TNF-α	17±2

4.5.2 Cytokine production in response to anti-CD3/CD28 monoclonal antibodies

Cell suspensions derived from human nasal polyps and stimulated with anti-CD3/CD28 monoclonal antibodies for 48 hours responded with a marked increase of all Th1/Th2 cytokines measured (Table 4.5.2).

The combined results for all subjects (3 atopics) are presented.

Table 4.5.2: Cytokine production following stimulation with anti-CD3/CD28 mAb. Cell suspensions derived from human nasal polyps were stimulated with anti-CD3/CD28 mAb and cultured for 48 hours. Cytokine production was assessed by CBA. (n=3) showing mean ± SEM in pg/mL

Cytokine	Mean ± SEM (pg/mL)
IL-10	181±61
IL-5	295±87
IFN-γ	2817±828
IL-2	134±49
IL-4	89±21
TNF-α	194±36

4.6 Discussion

Our initial experiments demonstrated a viable model for testing the cytokine production in cells derived from human nasal polyps. We confirmed the findings of previous researchers, that nasal polyp cell suspensions constitutively produce a mixed Th1/Th2 cytokine expression including IL-10, IL5 and IFN-γ (343-345). The addition of anti-CD3/CD28 monoclonal antibodies resulted in significant production of all cytokines measured, confirming the presence of functional T cells.

5 ALLERGEN-SPECIFIC CYTOKINE RESPONSE

5.1 Introduction

5.1.1 Human peripheral blood evidence

In humans, the cytokine response of allergen-specific peripheral blood T cells varies according to atopic status. Allergen-specific Th1, Th2 and Treg subsets and their associated cytokines are present in all individuals, but the relative proportions vary, with healthy individuals demonstrating a Treg/ IL-10 predominance and atopic individuals demonstrating a Th2 skewed cytokine profile (220).

Allergen-specific peripheral blood Treg cells, when exposed to allergen, can be induced *in vivo* and *in vitro* to secrete anti-inflammatory cytokines, particularly IL-10, thereby regulating immune responses (108, 131, 346, 347). Indeed, IL-10 levels in the circulation have been demonstrated to be reduced in allergic individuals compared to non-atopic controls (115, 220, 228), and increased in such individuals when successfully treated with immunotherapy or glucocorticoids (115, 118, 121, 231, 348, 349).

5.1.2 Human respiratory tissue evidence

The evidence regarding the immunomodulatory cytokine response to specific allergen in human respiratory tissue (as opposed to the peripheral blood) from atopic and non-atopic individuals is yet to be comprehensively established.

Ex vivo bronchial biopsies from atopic asthmatics responded to HDM allergen stimulation with increased IL-5 by ELISA, after 24 hours culture, whereas atopic, non-asthmatics did not (97). Similar results have been seen with bronchoalveolar lavage (350, 351) and nasal biopsies in AR (352).

There is limited human evidence to suggest that tissue-resident IL-10 producing T regulatory cells are important in limiting allergic inflammation at mucosal sites (97, 110, 234). Much of this evidence is derived indirectly from studies of bronchoalveolar lavage (236, 350), intranasal instillation of

cytokines (235), and the observation that treatments that improve disease symptoms, such as glucocorticoids and allergen immunotherapy are associated with increased CD4+CD25+ Tcells in the nasal tissue of subjects who responded to treatment (226). It remains unclear whether the Th2 skewed cytokine response to specific allergen seen in some atopics is a consequence of reduced Treg cell numbers or defective Treg cell function (353), or a third, less-likely possibility, that activation of effector T cells in atopics is immune to Treg cell suppression – ie the defect lies in the effector cells (106).

5.2 Aims

The aims of this experiment were:

To determine the effect of specific allergen on cytokine production by cells derived from human nasal polyps.

To determine whether such cytokine production varied according to atopic status and sensitisation status to the specific allergen used.

5.3 Hypotheses

We hypothesised that:

The addition of specific allergen to cell suspensions derived from human nasal polyps would result in increased regulatory IL-10 production, consistent with the clinically observed lack of allergic response to specific allergen in subjects with nasal polyps.

5.4 Methods

The methods used are detailed in Chapter 3.

Briefly, nasal polyps were harvested, processed, digested with collagenase and filtered to produce a single cell suspension that was seeded at 1 X 10^5 cells per well. Wells were incubated with and without allergen at 10^2 and 10^4

U/mL for 6 days and the cell supernatants harvested. CBA analysis was performed to determine cytokine levels.

5.5 Results

The results for all subjects (12 atopic and 8 non-atopic, 20 total) and all allergens (cat, grass, HDM) are presented for each cytokine.

Results are also subdivided according to atopic status (atopic and non-atopic). Results for atopics are further subdivided according to whether the allergen added was one that the subject was sensitised to, or not-sensitised to (ie the subject was atopic, but to a different allergen).

Cat allergen was added to 18 polyp cell suspensions (6 atopic and sensitised, 6 atopic but non-sensitised to cat and 6 non-atopic), grass allergen to 14 (7 atopic and sensitised, 2 atopic but non-sensitised to grass and 5 non-atopic) and HDM allergen to 14 (6 atopic and sensitised, 3 atopic but non-sensitised to HDM and 5 non-atopic). This gave a total of 46 sets of results for 20 subjects (Table 5.5.1).

Table 5.5.1: Number of results according to atopy and sensitisation status.

		Atopic		Non-atopic
		Sensitised to allergen added	Non-Sensitised to allergen added	
Allergen added	Cat	6	6	6
	Grass	7	2	5
	HDM	6	3	5

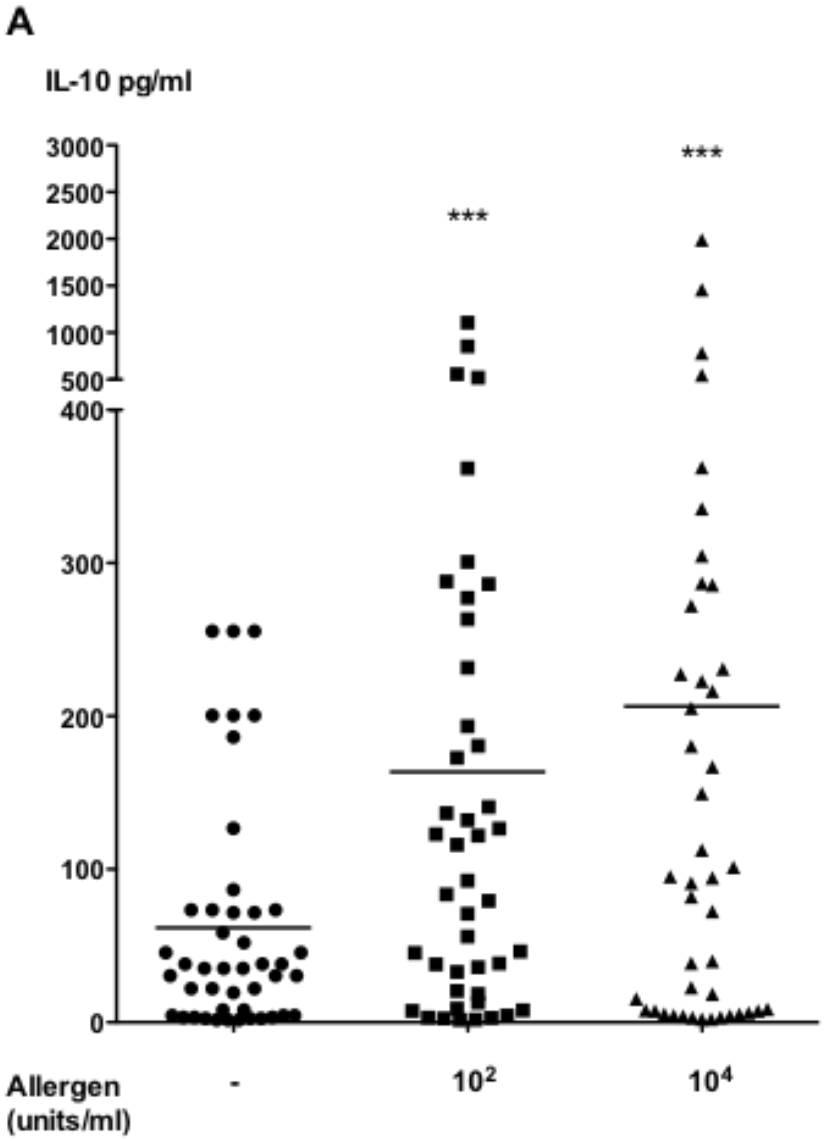
Note, that not all allergens were added to each cell suspension. For example, a non-atopic subject would have their cell suspension tested with either one, two or all three allergens.

Two different concentrations (10^2 U/mL and 10^4 U/mL) are presented for each of the sets of results.

5.5.1 IL-10

5.5.1.1 IL-10 Combined results

Allergen induced IL-10 production by human nasal polyp cell suspensions in a dose-response fashion (Figure 5.5.1).



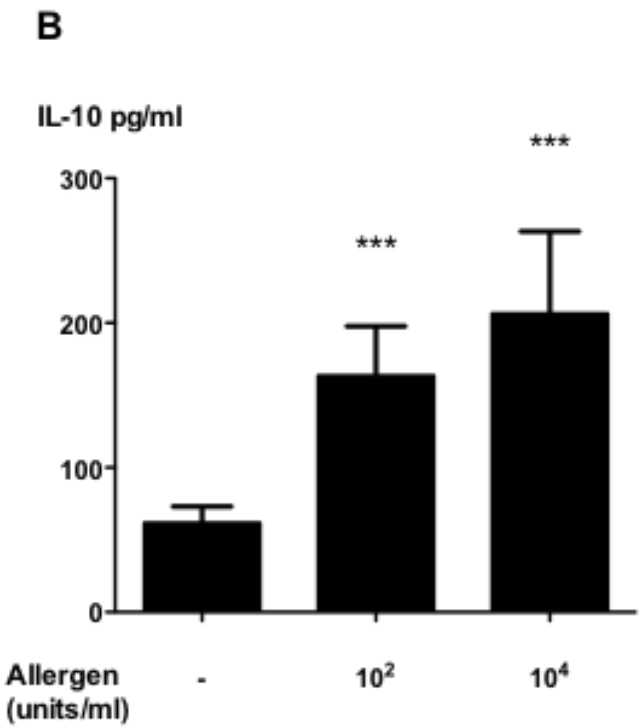


Figure 5.5.1: Production of IL-10 by polyp cell suspensions from atopic and non-atopic subjects following exposure to allergen. (A) Combined results for all subjects presented (n=20 subjects). Allergens added: cat (n=18), grass (n=14) or HDM (n=14) (46 sets of results for 20 subjects) at concentrations of 10^2 and 10^4 U/mL. (B) indicates mean ± SEM. *** denotes statistically significant difference from control (no allergen added); $p<0.005$ by Wilcoxon matched-pairs signed rank test.

5.5.1.2 IL-10 Results subdivided by atopic status

Human nasal polyp cell suspensions from atopic subjects produced increased IL-10 when exposed to allergen that the subject was sensitised to (Figure 5.5.2).

Human nasal polyp cell suspensions from atopic subjects did not significantly produce IL-10 when exposed to other allergens (Figure 5.5.2).

Human nasal polyp cell suspensions from non-atopic subjects did not significantly produce increased IL-10 when exposed to allergen, although there was a trend towards increased production (Figure 5.5.2).

The results for added cat and grass allergen are presented for all subjects (12 atopic and 8 non-atopic, 20 total). The results for added HDM allergen are not presented as they were inconsistent and did not demonstrate significance (see Appendix 2 – raw data and 5.6 Discussion).

Cat allergen was added to 18 polyp cell suspensions (6 atopic and sensitised, 6 atopic but non-sensitised to cat and 6 non-atopic), grass allergen to 14 (7 atopic and sensitised, 2 atopic but non-sensitised to grass and 5 non-atopic). This gave a total of 32 sets of results for 20 subjects.

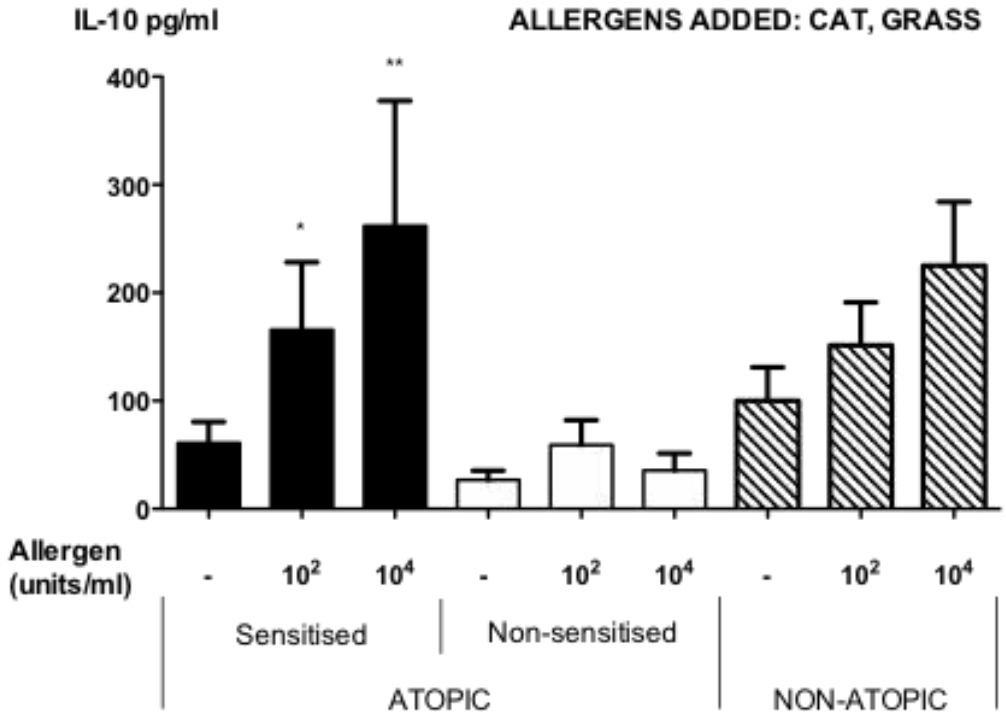


Figure 5.5.2: Production of IL-10 by polyp cell suspensions from atopic and non-atopic subjects following exposure to allergen. Results subdivided according to atopy (atopic and non atopic) and allergen sensitisation status (sensitised and non-sensitised) (n=20 subjects). Allergens added: cat (n=18), grass (n=14) (32 sets of results for 20 subjects) at concentrations of 10^2 and 10^4 U/ml showing mean ± SEM. *, ** denotes statistically significant difference from control (no allergen added); $p<0.05$, $p<.01$ by Wilcoxon matched-pairs signed rank test.

5.5.2 IL-5

5.5.2.1 IL-5 Combined results

The addition of allergen to human nasal polyp cell suspensions did not induce the production of IL-5 (Figure 5.5.3).

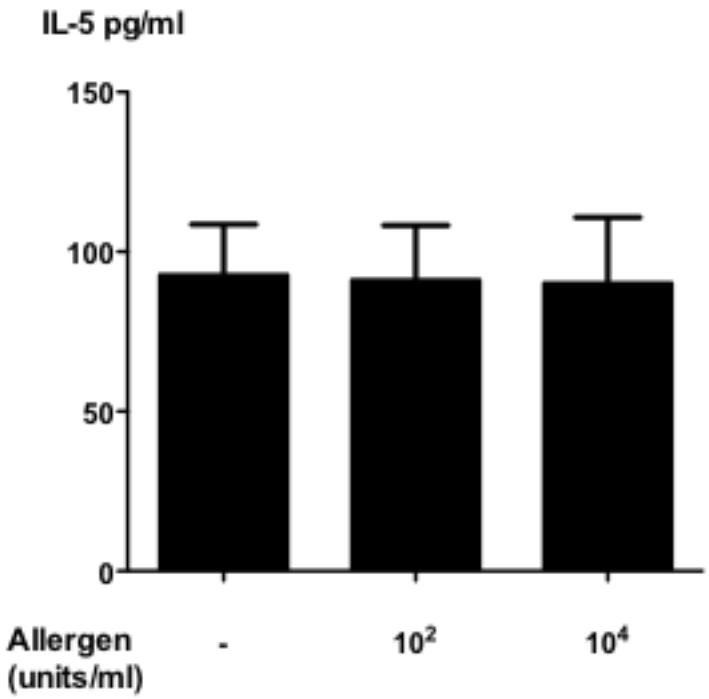


Figure 5.5.3: Production of IL-5 by polyp cell suspensions from atopic and non-atopic subjects following exposure to allergen. Combined results for all subjects presented (n=20 subjects). Allergens added: cat (n=18), grass (n=14) or HDM (n=14) (46 sets of results for 20 subjects) at concentrations of 10^2 and 10^4 units/mL showing mean ± SEM. No statistically significant difference from control (no allergen added) by Wilcoxon matched-pairs signed rank test.

5.5.2.2 IL-5 Results subdivided by atopic status

The addition of allergen to human nasal polyp cell suspensions did not induce the production of IL-5 in any of the subgroups analysed, although there was a non-significant trend towards increased IL-5 production in atopics with sensitised allergen (Figure 5.5.4).

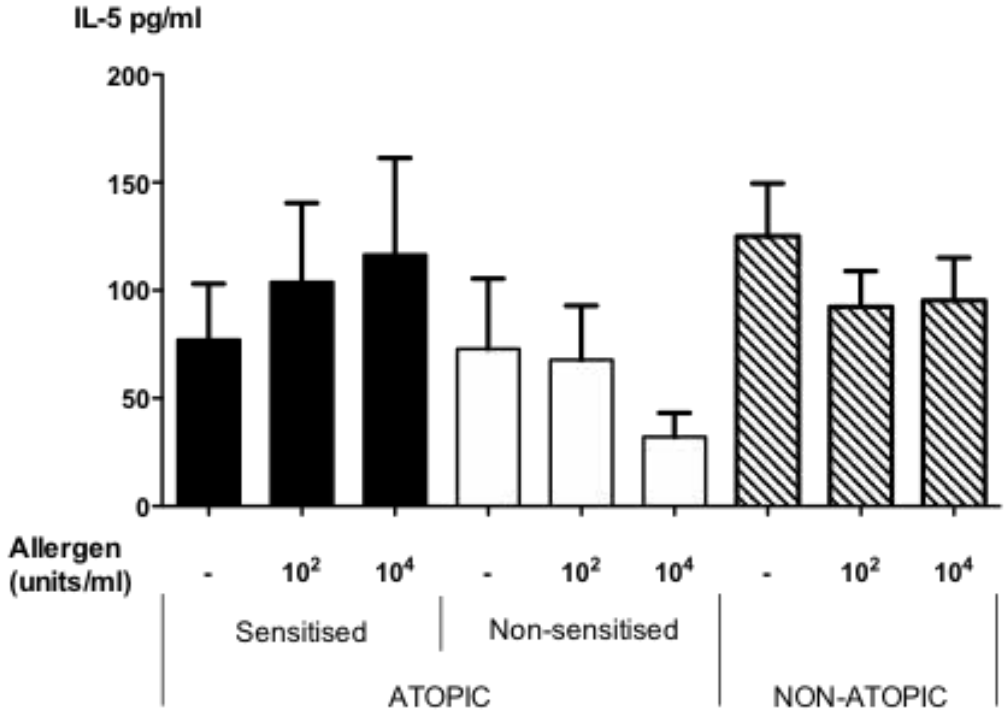


Figure 5.5.4: Production of IL-5 by polyp cell suspensions from atopic and non-atopic subjects following exposure to allergen. Results subdivided according to atopy (atopic and non atopic) and allergen sensitisation status (sensitised and non-sensitised) (n=20 subjects). Allergens added: cat (n=18), grass (n=14) or HDM (n=14) (46 sets of results for 20 subjects) at concentrations of 10^2 and 10^4 U/ml showing mean ± SEM. No statistically significant difference from control (no allergen added) by Wilcoxon matched-pairs signed rank test.

5.5.3 IFN-γ

5.5.3.1 IFN-γ Combined results

The addition of allergen to human nasal polyp cell suspensions did not induce the production of IFN-γ (Figure 5.5.5). There was a non-significant trend towards *decreased* production in the presence of allergen.

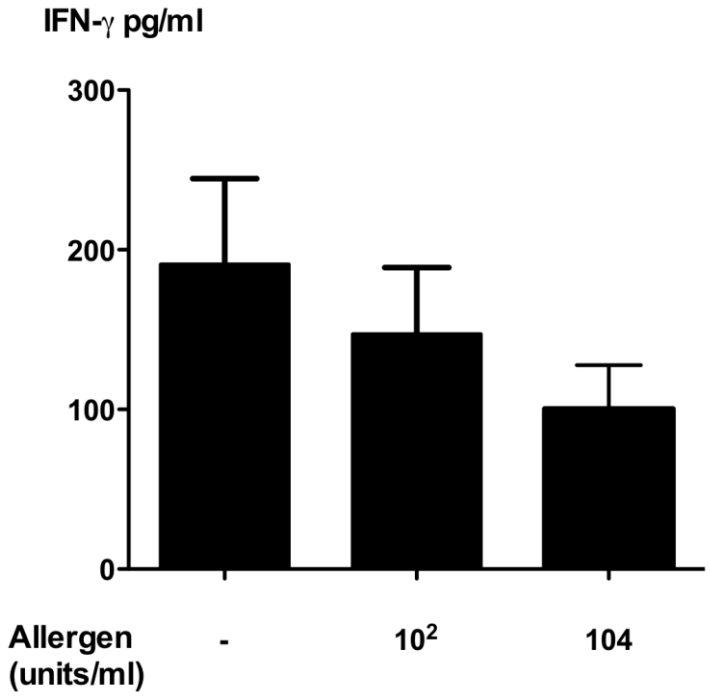


Figure 5.5.5: Production of IFN-γ by polyp cell suspensions from atopic and non-atopic subjects following exposure to allergen. Combined results for all subjects presented (n=20 subjects). Allergens added: cat (n=18), grass (n=14) or HDM (n=14) (46 sets of results for 20 subjects) at concentrations of 10^2 and 10^4 units/mL showing mean ± SEM. No statistically significant difference from control (no allergen added) by Wilcoxon matched-pairs signed rank test.

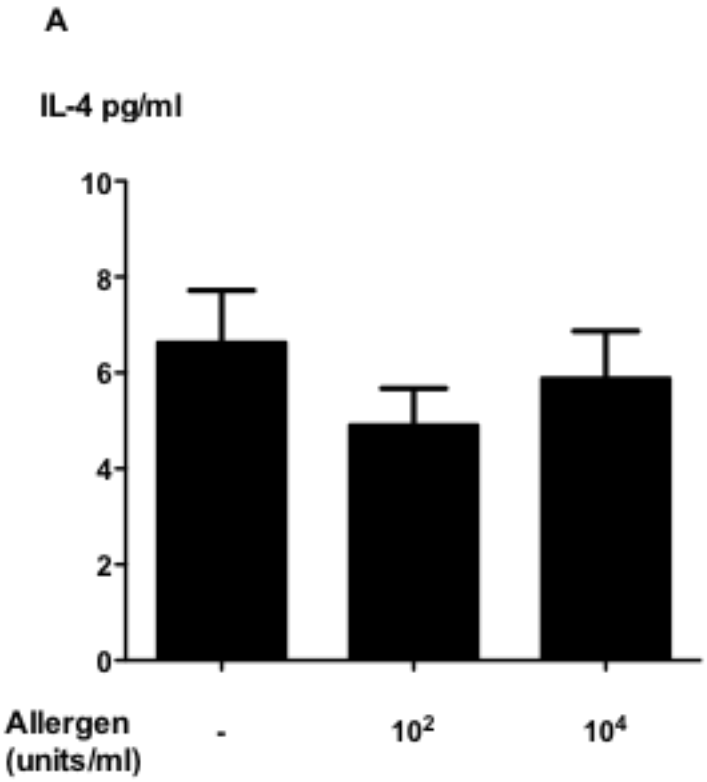
5.5.3.2 IFN-γ Results subdivided by atopic status

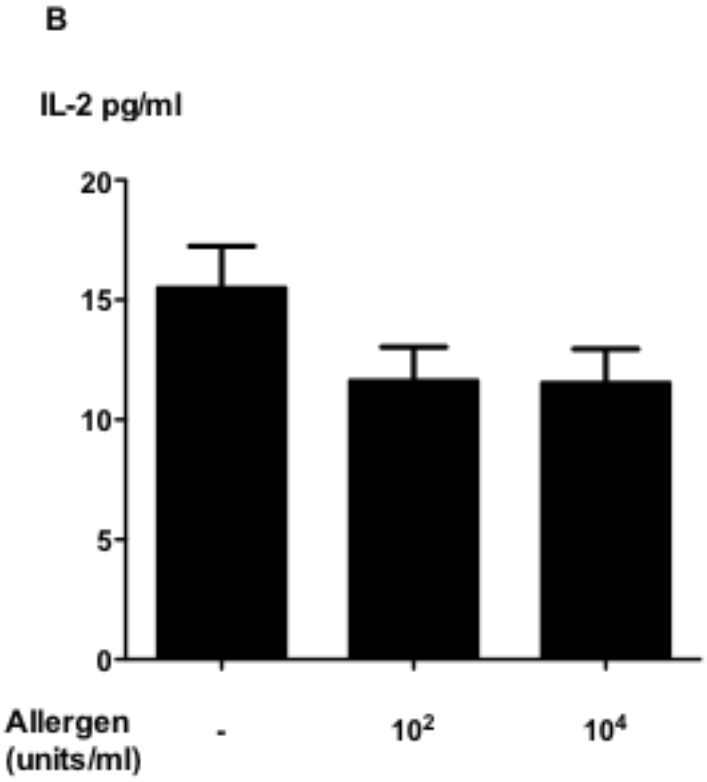
The addition of allergen to human nasal polyp cell suspensions did not induce the production of IFN-γ in any of the subgroups analysed (atopic sensitised, atopic non-sensitised and non-atopic) (data not shown).

5.5.4 IL-4, IL-2 and TNF-α

5.5.4.1 IL-4, IL-2 and TNF-α Combined results

The addition of allergen to human nasal polyp cell suspensions did not induce the production of IL-4, IL-2 or TNF-α, with the amount produced remaining barely detectable (Figure 5.5.6).


A
IL-4 pg/ml
10
8
6
4
2
0
Allergen (units/ml)
-
10^2
10^4


B
IL-2 pg/ml
20
15
10
5
0
Allergen (units/ml)
-
10^2
10^4

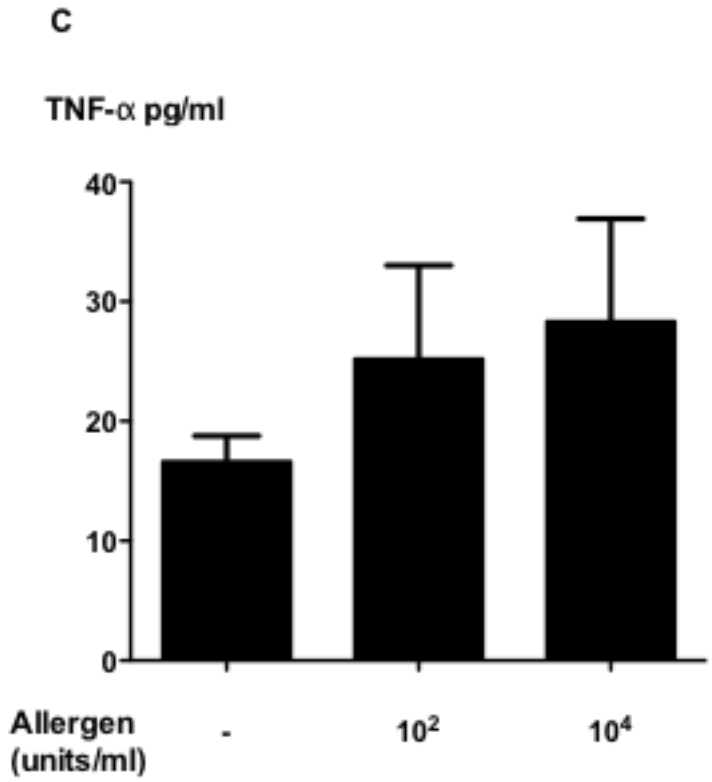


Figure 5.5.6: Production of (A) IL-4, (B) IL-2 and (C) TNF-α by polyp cell suspensions from atopic and non-atopic subjects following exposure to allergen. Combined results for all subjects presented (n=20 subjects). Allergens added: cat (n=18), grass (n=14) or HDM (n=14) (46 sets of results for 20 subjects) at concentrations of 10^2 and 10^4 units/mL showing mean ± SEM. No statistically significant difference from control (no allergen added) by Wilcoxon matched-pairs signed rank test.

5.5.5 Combined results for all cytokines

To facilitate comparison of absolute values, Figure 5.5.7 demonstrates all measured cytokines, including constitutive, allergen-specific (at one of the two measured concentrations; 10^4 U/mL) and anti-CD3/CD28 mAb-stimulated production, using similar axes.

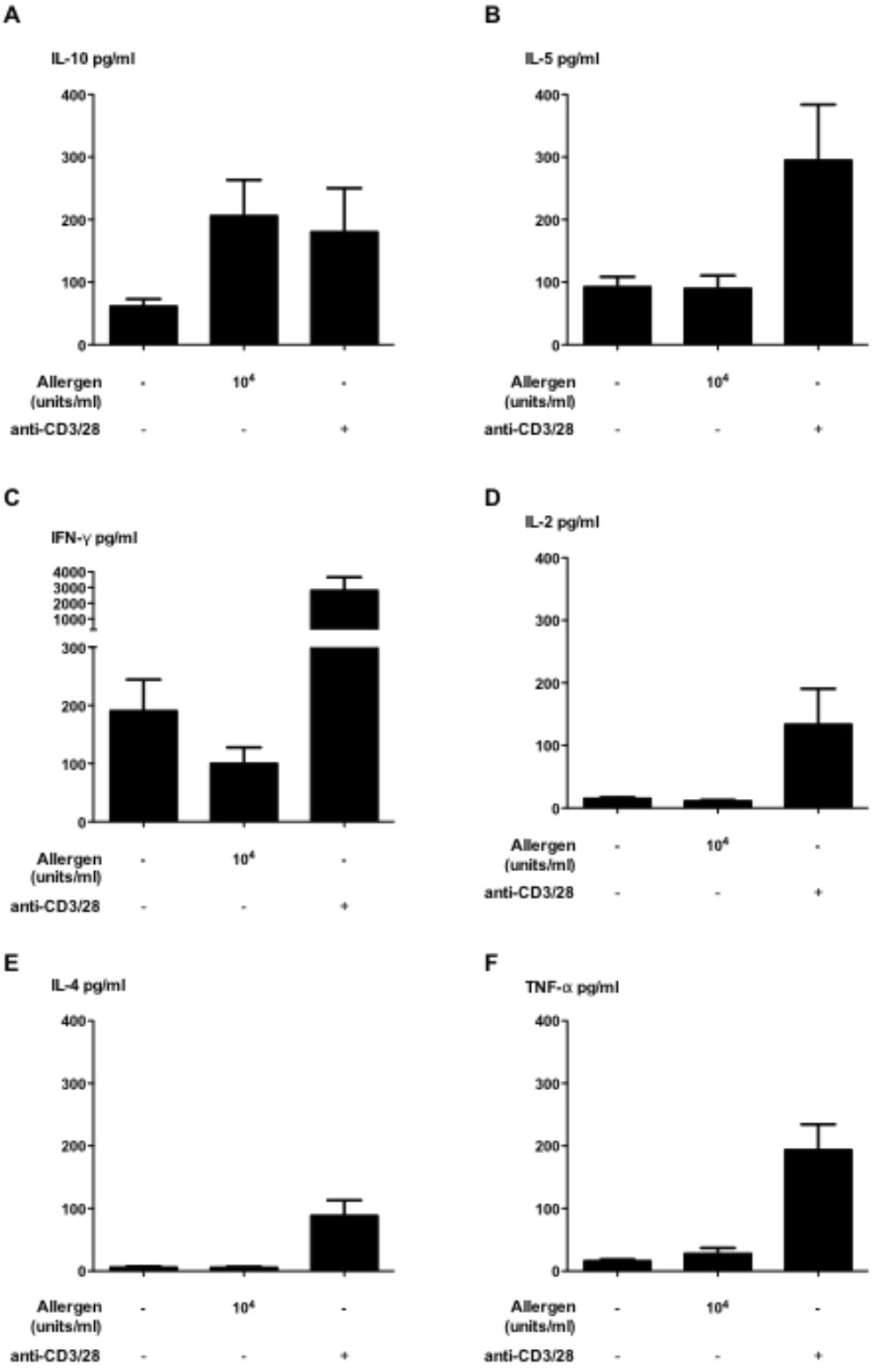


Figure 5.5.7: Constitutive, Allergen-specific (10^4) and anti-CD3/CD28 mAb-stimulated production of (A) IL-10, (B) IL-5, (C) IFN-γ, (D) IL-2, (E) IL-4 and (F) TNF-α showing mean ± SEM. Constitutive and allergen-specific production showing combined results for all subjects (n=20) at 6 days. Allergens added: cat (n=18), grass (n=14) or HDM (n=14) (46 sets of results for 20 subjects) at concentration of 10^4 units/mL. Anti-CD3/CD28 antibody-stimulated production showing results for n=3 subjects at 48 hours.

5.6 Discussion

5.6.1 Summary of key findings

The addition of specific allergen to *ex vivo* human nasal polyp cell suspensions results in increased IL-10 production, in a dose-response fashion, particularly where the allergen added is one the subject is sensitised to. By contrast, production of IL-5 and IFN-γ does not increase.

These results imply an IL-10 dependent local suppression of Th2 and Th1 responses to specific allergen in nasal polyp tissue.

5.6.2 IL-10

The addition of specific allergen resulted in a predictable, dose-response increase in the production of IL-10. The effect was marked and statistically significant.

The IL-10 produced was induced by *ex vivo* cells, implying local mucosal tissue based responses, without the need for draining lymph node processing or peripheral blood cell interactions.

Upon subdividing the results, the significant response associated with sensitised allergens and the weak response associated with non-sensitised allergens may represent the higher percentage of immune cells specific to the sensitised allergen in these subjects' cell suspensions.

The addition of HDM allergen demonstrated an unexpected result, compared to cat and grass allergen. Stimulation with HDM allergen resulted in a marked, but non-statistically-significant, increase in IL-10 production in non-HDM-sensitised atopic subjects. The number of data points in this subgroup was insufficient (n=3) to reach statistical significance and allow any meaningful conclusions to be drawn. However, the results were intriguing and may possibly be explained by the unusual mechanisms of HDM allergy.

Allergy to HDM is mediated by the typical "adaptive" Th2 pathway, involving IgE mediated recruitment of inflammatory mediators. However, unlike other

allergens, HDM can also elicit allergic reactions via the “innate” immune response. *Der p1*, in addition to being an antigenic protein, is also a protease (354) and has been demonstrated to directly activate airway epithelial cells to secrete pro-inflammatory, pro-Th2 cytokines (30). It is possible that this mechanism may be related to the observed response in non-HDM-sensitised atopics to stimulation with HDM allergen.

5.6.3 IL-5

Ex vivo human nasal polyp cell suspensions do not respond to the addition of allergen with the production of IL-5. Despite the presence of constitutive IL-5 production, there appears to be a regulatory mechanism in place, limiting the Th2 response to allergen stimulation.

This correlates with the observed clinical findings that nasal polyps do not respond to allergen exposure with typical allergic responses clinically or with the production of immune mediators (240, 276). It has been noted that *ex vivo* bronchial explants in atopic, non-asthmatics do not respond to added allergen with IL-5 production, whereas in atopic asthmatics, IL-5 is produced (97). Gevaert *et al* have proposed that this lack of response in nasal polyps may be due to locally produced polyclonal IgE, which saturates Fcε receptors on mast cells, limiting the ability of added allergen to stimulate a response (276). Polyclonal IgE production may also limit the capacity for specific antibody synthesis (276). However, polyclonal IgE has only been demonstrated in around 50% of Western NP patients (276). Therefore, another more universal regulatory mechanism appears to be in place.

5.6.4 IFN-γ

Ex vivo human nasal polyp cell suspensions do not respond to the addition of allergen with the production of IFN-γ. As observed with IL-5, despite the presence of constitutive IFN-γ production, there appears to be a regulatory mechanism in place, limiting the Th1 response to allergen stimulation.

5.6.5 IL-4, IL-2 and TNF-α

Ex vivo human nasal polyp cell suspensions do not constitutively produce appreciable quantities of IL-4, IL-2 and TNF-α and do not respond to the addition of allergen with the production of increased IL-4, IL-2 and TNF-α by CBA. Stimulation with anti-CD3/CD28 mAbs did result in increased production of IL-4, IL-2 and TNF-α demonstrating the presence of viable T cells with the functional capacity to produce these cytokines.

6 REGULATION OF IL-5, IFN-γ AND TNF-α BY IL-10

6.1 Introduction

In mucosal sites, such as the lung (127-129) and gut (130), it has been suggested that IL-10 plays a critical role in suppressing pathologic Th2 and Th1 responses to allergen.

IL-10 levels have been demonstrated to be reduced in pathologic states and elevated in health (115, 116, 228, 229). Patients undergoing successful treatment with allergen immunotherapy and glucocorticoids have been demonstrated to have raised IL-10 levels as their tolerance to allergen improves (115, 118, 121, 131, 231, 348, 349). This has largely been seen in animal tissue, human peripheral blood and indirectly in human respiratory tissue, however there is a limited amount of corroborating evidence from human nasal tissue (122).

These findings suggest that IL-10 has a regulatory role in suppressing abnormal responses to allergen.

6.2 Aims

The aim of this experiment was to determine whether IL-10 suppresses IL-5 and IFN-γ production, as representatives of Th2 and Th1 type responses, respectively, in human nasal polyp cell suspensions.

6.3 Hypotheses

We hypothesised that neutralising IL-10 in human nasal polyp cell suspensions would result in increased production of IL-5 and IFN-γ in response to allergen.

6.4 Methods

The methods used are detailed in Chapter 3.

Briefly, nasal polyps were harvested, processed, digested with collagenase and filtered to produce a single cell suspension that was seeded at 1 X 10^5 cells per well. Anti-IL-10 antibodies were added to non-control wells. Rat IgG1 was used as an isotype control against anti-IL-10 antibodies in some experiments. Wells were incubated with and without allergen at 10^2 and 10^4 U/mL for 6 days and the cell supernatants harvested. CBA analysis was performed to determine cytokine levels.

6.5 Results

Polyp cell suspensions from 13 subjects (8 atopic and 5 non-atopic) were used, with 1-3 allergens added, for a total of 22 sets of results. A concentration-matched isotype control antibody, Rat IgG1, was used during initial experiments (n=6) and had no effect upon production of any of the cytokines tested.

Cat allergen was added to 8 polyp cell suspensions (5 atopic and 3 non-atopic), grass allergen to 7 (3 atopic and 4 non-atopic) and HDM allergen to 7 (3 atopic and 4 non-atopic). This gave a total of 22 sets of results for 13 subjects

6.5.1 Effect of addition of anti-IL-10 antibodies on IL-10 production

The addition of neutralising anti-IL-10 antibodies to human nasal polyp cell suspensions stimulated with allergen resulted in almost complete ablation of IL-10 production, as expected (Figure 6.5.1).

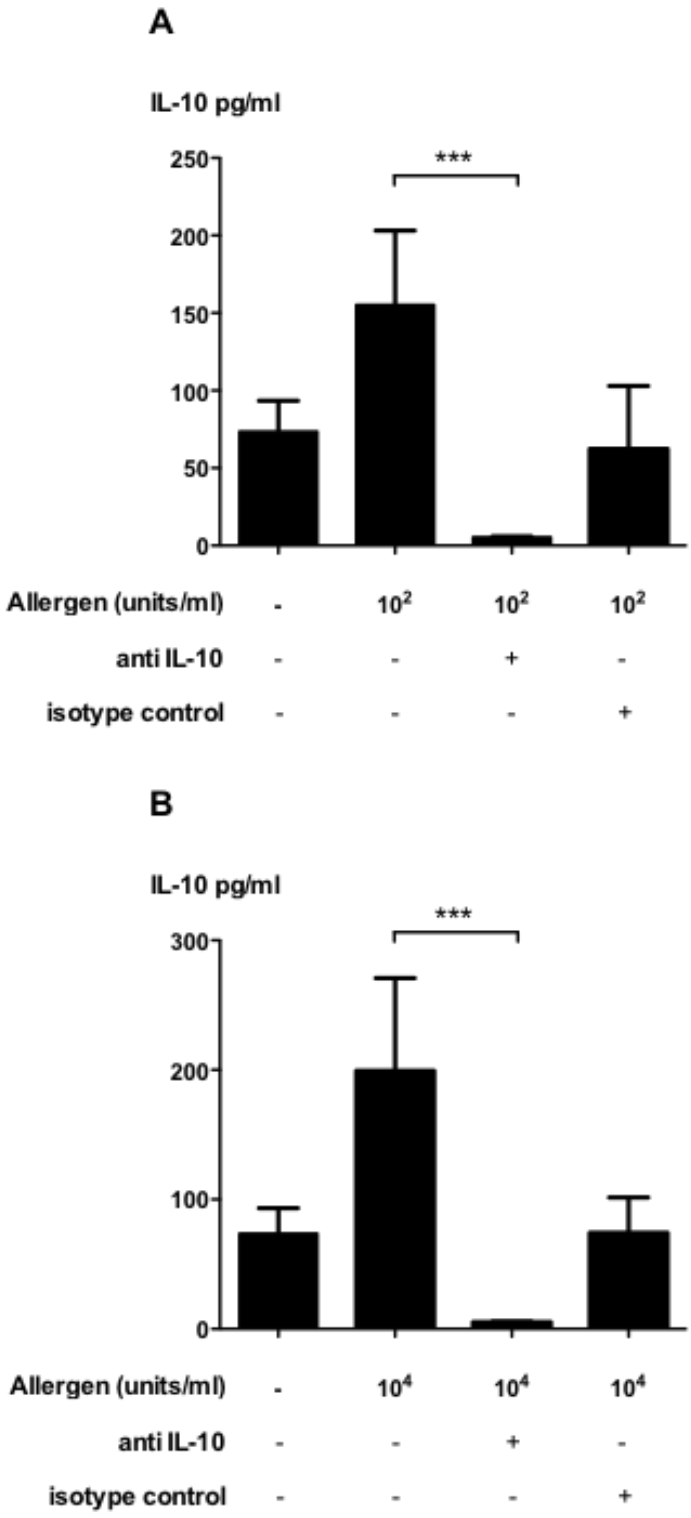


Figure 6.5.1: Allergen-specific IL-10 production by polyp cell suspensions from atopic and non-atopic subjects. Combined results for all subjects presented. Allergen (cat, grass, HDM) added at (A) 10^2 and (B) 10^4 units/mL (n=13 subjects with 1-3 allergens added for 22 sets of results for 10^2, 10^2 + anti-IL-10, 10^4 and 10^4 + anti-IL-10; n=6 with 1 allergen added for 6 sets of results for 10^2 + isotype control and 10^4 + isoptype control). Mean ± SEM. *** denotes statistically significant difference from sample with allergen added but without added anti IL-10 (*** $p < 0.005$ by Wilcoxon matched-pairs signed rank test).

6.5.2 Effect of addition of anti-IL-10 antibodies on IL-5 production

The addition of anti-IL-10 antibodies was associated with a significant induction of IL-5 production (Figure 6.5.2).

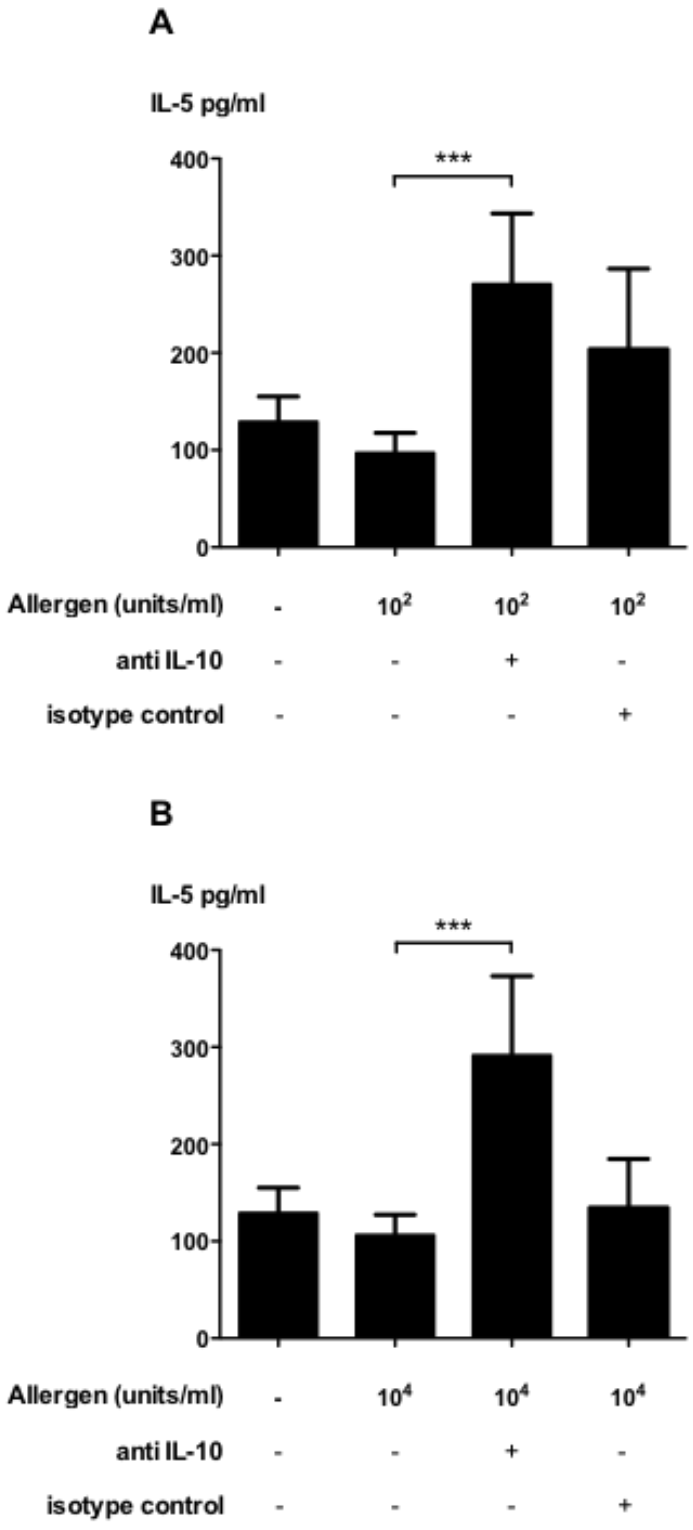


Figure 6.5.2: Allergen-specific IL-5 production by polyp cell suspensions from atopic and non-atopic subjects. Combined results for all subjects presented. Allergen (cat, grass, HDM) added at (A) 10^2 and (B) 10^4 units/mL (n=13 subjects with 1-3 allergens added for 22 sets of results for 10^2, 10^2 + anti-IL-10, 10^4 and 10^4 + anti-IL-10; n=6 with 1 allergen added for 6 sets of results for 10^2 + isotype control and 10^4 + isoptype control). Mean ± SEM. *** denotes statistically significant difference from sample with allergen added but without added anti IL-10 (*** $p < 0.005$ by Wilcoxon matched-pairs signed rank test).

6.5.3 Effect of addition of anti-IL-10 antibodies on IFN-γ production

The addition of anti-IL-10 antibodies also resulted in the induction of IFN-γ at one of the two concentrations (10^4 units/mL). At the lower concentration (10^2 units/mL), the result approached significance at p=0.06 (Figure 6.5.3).

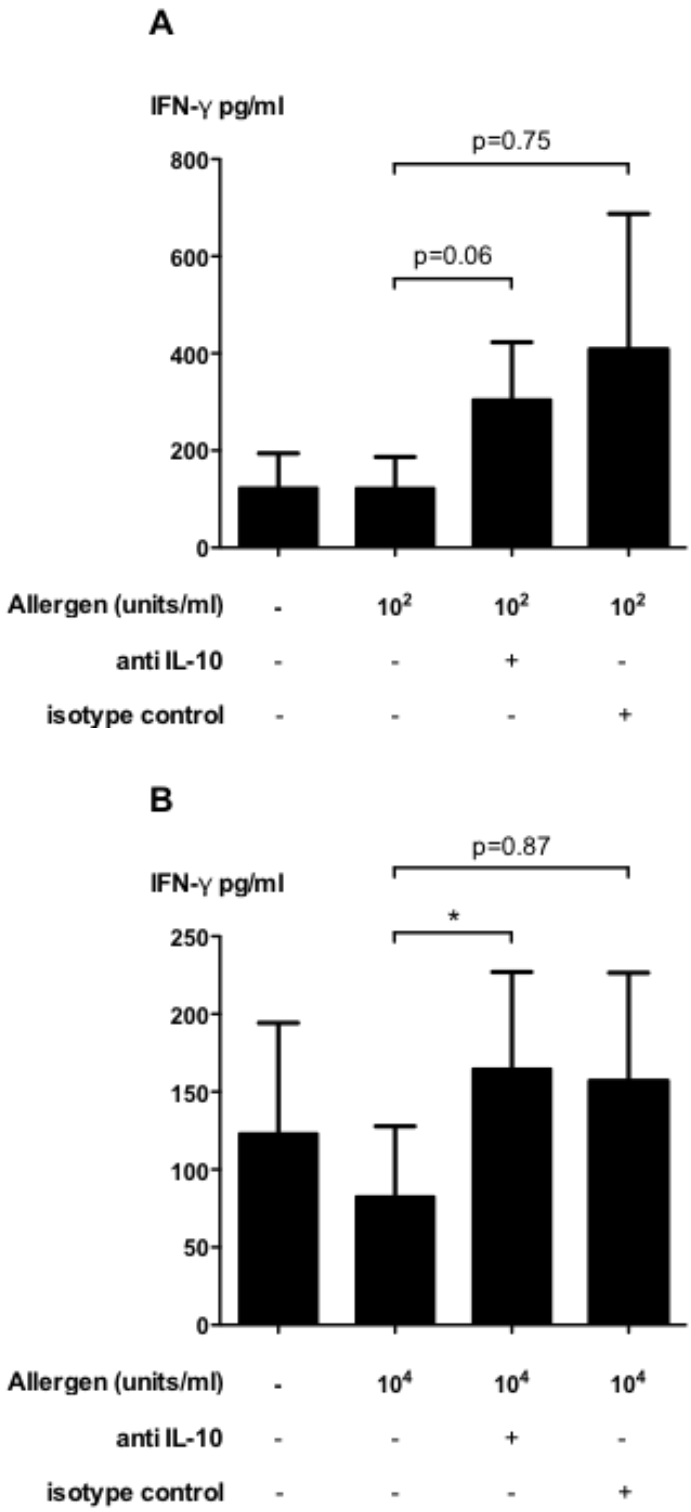


Figure 6.5.3: Allergen-specific IFN-γ production by polyp cell suspensions from atopic and non-atopic subjects. Combined results for all subjects presented. Allergen (cat, grass, HDM) added at (A) 10^2 and (B) 10^4 units/mL (n=13 subjects with 1-3 allergens added for 22 sets of results for 10^2, 10^2 + anti-IL-10, 10^4 and 10^4 + anti-IL-10; n=6 with 1 allergen added for 6 sets of results for 10^2 + isotype control and 10^4 + isoptype control). Mean ± SEM. * denotes statistically significant difference from sample with allergen added but without added anti IL-10 (* $p < 0.05$ by Wilcoxon matched-pairs signed rank test).

6.5.4 Effect of addition of anti-IL-10 antibodies on TNF-α production

The addition of anti-IL-10 antibodies was associated with a significant induction of TNF-α at both concentrations tested (Figure 6.5.4).

There was an outlier (Experiment 24). Following exclusion of the outlier (not shown), the result at the weaker concentration (10^2) remained statistically significant, whereas the result at the stronger concentration (10^4) did not reach significance (p=0.052) (not shown).

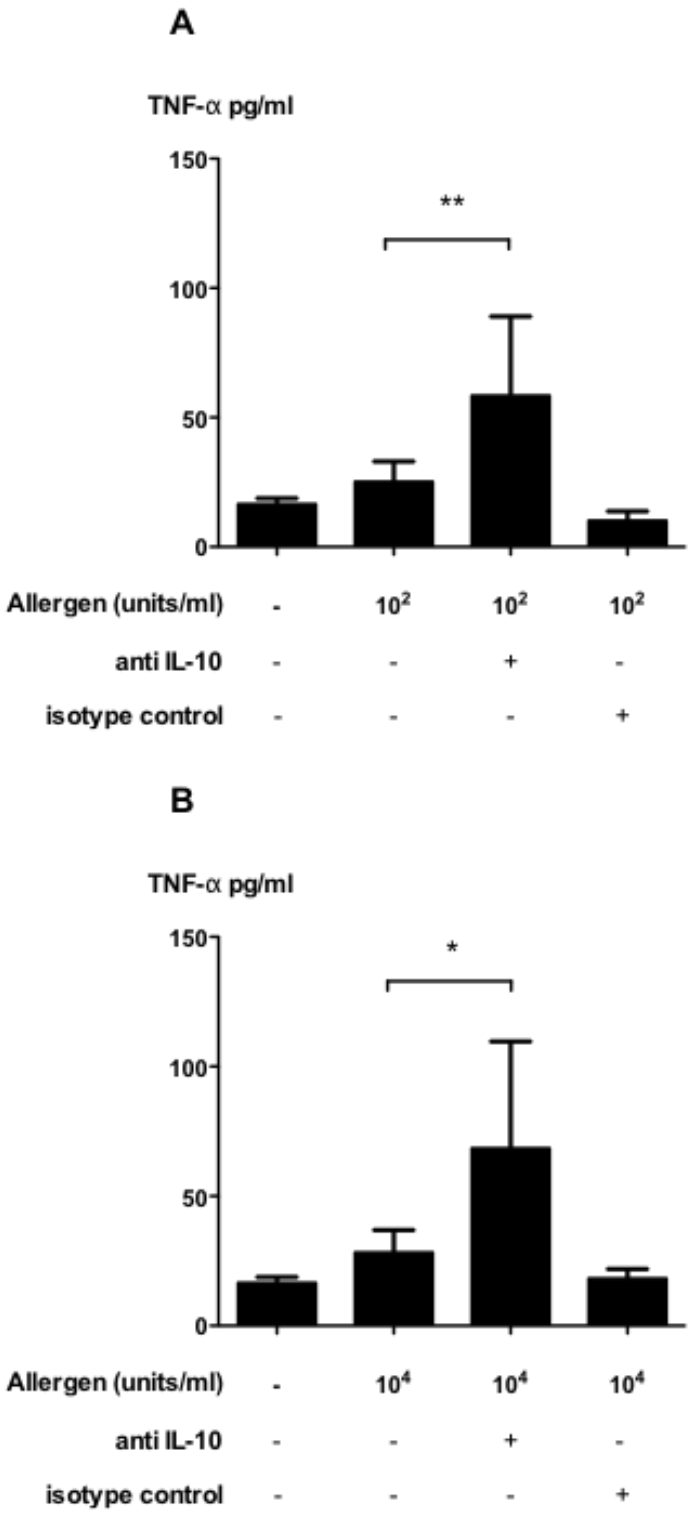


Figure 6.5.4: Allergen-specific TNF-α production by polyp cell suspensions from atopic and non-atopic subjects. Combined results for all subjects presented. Allergen (cat, grass, HDM) added at (A) 10^2 and (B) 10^4 units/mL (n=13 subjects with 1-3 allergens added for 22 sets of results for 10^2, 10^2 + anti-IL-10, 10^4 and 10^4 + anti-IL-10; n = 6 with 1 allergen added for 6 sets of results for 10^2 + isotype control and 10^4 + isoptype control). Mean ± SEM. **, * denotes statistically significant difference from sample with allergen added but without added anti IL-10 (** $p < 0.01$, * $p<0.05$ by Wilcoxon matched-pairs signed rank test).

6.5.5 Effect of addition of anti-IL-10 antibodies on IL-2 and IL-4 production

The addition of anti-IL-10 antibodies was not associated with any significant change in production of IL-2 or IL-4 (not shown).

6.6 Discussion

When IL-10 was neutralised with anti-IL-10 antibodies, there was a significant increase in IL-5 and, to a lesser extent, IFN-γ and TNF-α production in response to allergen exposure. The earlier experiments demonstrated that, in response to allergen exposure, production of these cytokines is not increased compared to constitutive production, whereas IL-10 production is increased.

The current experiments indicate that IL-10 normally has a suppressive effect upon Th2/ Th1 cytokine production in response to allergen. When this suppressive effect is removed, human nasal polyp cell suspensions respond by producing significant quantities of IL-5 and, to a lesser extent, IFN-γ and TNF-α.

This suggests a critical regulatory role for IL-10 in suppressing the Th2 and Th1 pathways in response to allergen exposure in human respiratory tissue.

This role has been demonstrated *in vivo* in mice, where IL-10 administration before allergen treatment induced antigen-specific peripheral blood T-cell unresponsiveness (355).

The role of IL-10 has also been demonstrated to be critical within murine mucosal surfaces, such as the lung (127-129) and gut (130), in order to suppress allergic responses.

In human peripheral blood, IL-10 levels are increased in atopic subjects successfully treated with immunotherapy and glucocorticoids (115, 118, 120, 121, 230, 231), as well as in healthy beekeepers, with a history of multiple bee-stings (356). *Ex vivo* neutralisation of IL-10 in PBMC cultures from such immunotherapy treated (118) and bee-keeping individuals (356), resulted in T cell proliferation and cytokine production typical of an allergic response (115).

Our experiments demonstrate a similar critical functional role for IL-10 in regulating the Th2 and Th1 responses to allergen exposure in *ex vivo* nasal polyp derived human respiratory tissue (as distinct from peripheral blood).

7 ORIGIN OF OBSERVED IL-10

7.1 Introduction

Having established that human nasal polyp cell suspensions produce IL-10 when exposed to allergen, we sought to determine the cell origin of this observed cytokine.

Nasal polyp cell suspensions are known to contain epithelial cells, leucocytes (T cells, B cells, mast cells, eosinophils, neutrophils, monocytes/ macrophages) and other cells (280). Table 8.2.1 indicates the composition of polyp cell suspensions by flow cytometry, carried out in our laboratory, subsequent to the experiments referred to in this thesis (see *8.2 Composition of nasal polyp cell suspensions*).

IL-10 is known to be secreted by Treg cells. However it is also produced by B cells, mast cells, macrophages, eosinophils, epithelial cells and certain APCs (357). Other T cells, including CD8+ and effector CD4+ Th1, Th2 and Th17 cells may produce IL-10 (358, 359).

7.2 Aims

We sought to determine the cell origin of the observed IL-10, produced when human nasal polyp cell suspensions were exposed to allergen.

7.3 Hypotheses

We hypothesised that the observed IL-10 was either being produced by T cells or via a T cell dependent process and that T cell depletion would result in a significant reduction in allergen-specific IL-10 production.

7.4 Methods

The methods used are detailed in Chapter 3.

Briefly, nasal polyps were harvested, processed, digested with collagenase and filtered to produce a single cell suspension.

In 2 experiments, T cells were depleted from the cell suspension prior to the addition of allergen, using a magnetic column. FACS analysis demonstrated >95% depletion of CD3+ T cells by this procedure (Figure 3.6.1). Controls were “mock depleted” by passing them through the column without depletion.

Wells were seeded at 1 X 10^5 cells per well and incubated with and without allergen at 10^2 and 10^4 U/mL for 6 days and the cell supernatants harvested. CBA analysis was performed to determine cytokine levels.

7.5 Results

There was a significant decrease in IL-10 production from cell suspensions following T cell depletion (Figure 7.5.1). Polyp cell suspensions from 2 subjects (1 atopic and 1 non-atopic) were used, with 3 allergens added, for a total of 6 sets of results

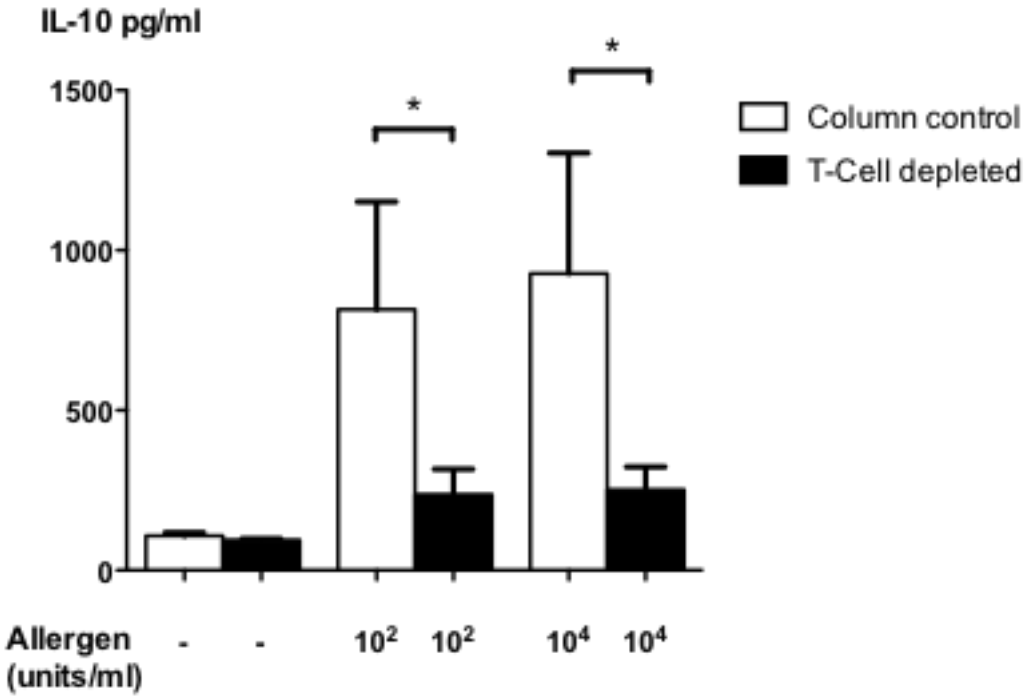


Figure 7.5.1: Allergen specific IL-10 production by polyp cell suspensions following depletion of T cells, showing mean ± SEM. T cells were depleted on a MACS column and IL-10 production in response to allergen determined. Results are also shown for a column control (see *3.6 Depletion of T cells*). Allergen added at 10^2 and 10^4 units/mL. Subjects n=2 with 3 added allergens for a total of 6 sets of results.* denotes statistically significant difference between column controls and T-cell depleted cell suspensions (* $p < 0.05$ by Wilcoxon matched-pairs signed rank test)

7.6 Discussion

Following depletion of T cells from human nasal polyp cell suspensions, there was a significant reduction in IL-10 production with allergen exposure.

These results indicate that the observed IL-10 is produced either directly by T cells or via a T-cell dependent process.

Several respiratory tissue cell types have been identified as being capable of producing IL-10, including mast cells (360), macrophages (131) and B cells (361). All of these cell types are present within nasal polyp cell suspensions (see *8.2 Composition of nasal polyp cell suspensions*). Even if these cells are a significant source of the observed IL-10, our results indicate that such IL-10 production requires signalling from allergen-activated T cells.

Despite this, we propose that the principal source of the observed IL-10 is likely to represent allergen-specific T cells within human nasal polyp cell suspensions (see *8.1 Intracellular cytokine production*). This may include both Treg populations and effector T cell subsets. Indeed, it has been demonstrated that CD4+CD25+ Treg cells can anergize CD4+CD25- T cells then recruit the same anergized cells to produce IL-10 (359). This suggests that Treg cells initiate the production of IL-10, and then recruit bystander T cells to perform significant IL-10 synthesis.

8 SUBSEQUENT EXPERIMENTS

Following the completion of the experiments performed for this thesis, the same laboratory undertook further experiments to continue this area of investigation. Whilst not a part of this thesis, these results are briefly presented, where relevant to the final conclusions.

8.1 Intracellular cytokine production

To further confirm the T cell origin of the observed IL-10, intracellular cytokine staining was performed on polyp cell suspensions from 5 subjects exposed to allergen. In these experiments the viable lymphocyte population (R1; Figure 8.1.1), initially gated on forward and side scatter characteristics, was further gated into CD3+ (R2) and CD3- (R3) populations (Figure 8.1.1 i). T cells immunoreactive for IL-10 were identified (Figure 8.1.1), albeit in low numbers (2 + 0.5 %), and were largely confined to the CD3+ population (Figure 8.1.1ii and iii). These cells did not express immunoreactivity for IL-2 (Figure 8.1.1ii) or IL-13 (data not shown).

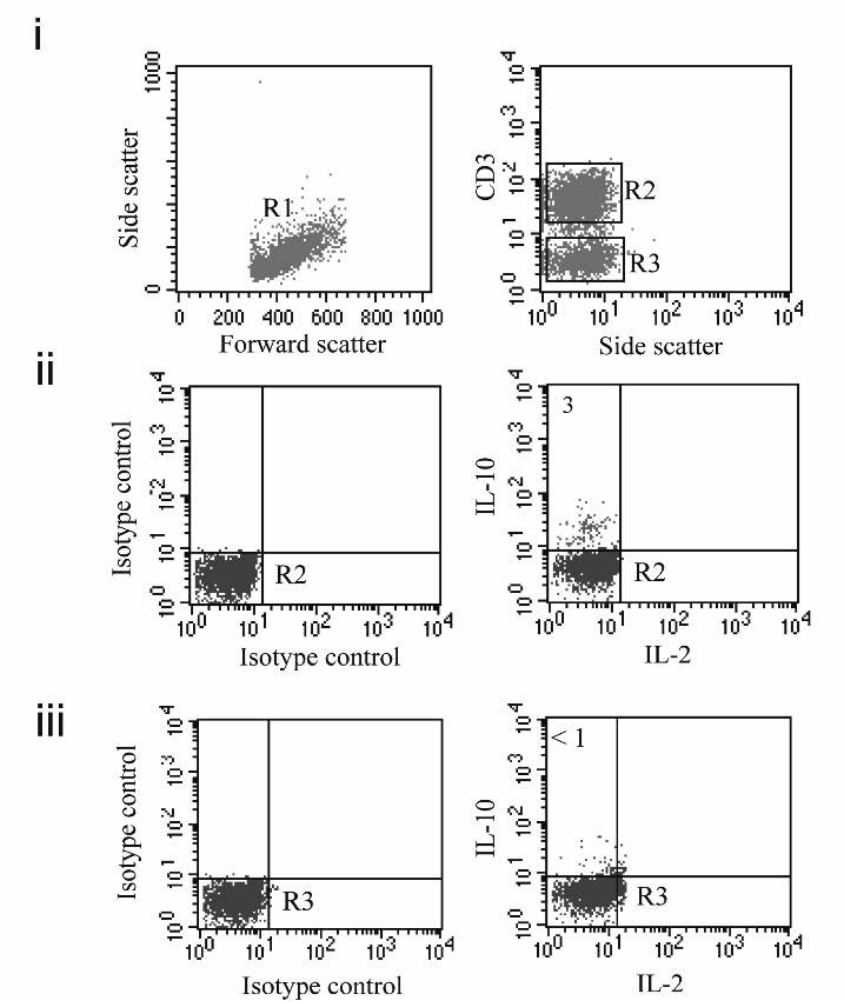


Figure 8.1.1: Polyp cells were stimulated with allergen for 4 days and immunophenotyed. (i) Viable lymphocytes were gated based on size and granularity (R1), and $CD3^+$ (R2) and $CD3^-$ (R3) cells selected. (ii) $CD3^+$ and (iii) $CD3^-$ cells were immunostained for intracellular production of IL-10 and IL-2. Figures represent % of total cells immunoreactive for IL-10. A representative experiment of 5 is shown.

These results confirm that, within the lymphocyte population, the observed IL-10 was found intracellularly, predominantly within T cells. The T cells identified were not Th1 or Th2 type cells, as indicated by the low expression of IL2 or IL13. These results imply the observed intracellular IL-10 was derived from either a regulatory T cell subset, or anergized bystander T cells, recruited to produce IL-10 (359).

8.2 Composition of nasal polyp cell suspensions

Flow cytometry was performed to determine the composition of nasal polyp cell suspensions. The suspensions contained CD45+ leucocytes (43.3%), CD326+ epithelial cells (19.2%) and other cells (Table 8.2.1).

Table 8.2.1 Phenotypes of nasal polyp cell suspensions

Cell type	**Marker**	**Percentage**[a]
Leucocytes	CD45+	43.3±7.0
Epithelial cells	CD326+	19.2±3.5
Other cells	CD45- CD326-	18.1±8.0

[a]Mean ± SEM

The CD45+ leucocytes were further subdivided into eosinophils, T (CD3+) and B (CD19+) lymphocytes, monocytes/macrophages (CD14+) and mast cells (CD117+) (Table 8.2.2).

Table 8.2.2 Phenotypes of leucocyte subsets in nasal polyp cell suspensions

Cell type	**Marker**	**Percentage[a]**
Eosinophils	CD45+ CD16- $CD14^{int}$ side $scatter^{hi}$	44.2±4.1
T lymphocytes	CD45+ CD3+	27.7±3.9
B Lymphocytes	CD45+ CD19+	8.7±2.4
Monocytes/ Macrophages	CD45+ CD14+	9.7±1.5
Mast Cells	CD45+ CD117+	8.0±2.1

[a]frequency within the leucocyte population

The CD3+ T cells were further subdivided into cytotoxic (CD8+) or presumed helper (CD8-). CD4 expression was lost during collagenase processing and hence could not be directly tested for. CD4 expression was restored on T cells following 36 hours of culture (not shown). Natural Treg cells (Foxp3+) comprised less than 3% of the T cell population. CD127 (133) has been proposed as a marker for non-Treg T cells in the peripheral blood. CD103 is a mucosal adhesion marker that may be found on mucosal Treg cells along with other cells. A significant percentage of the observed T cells were CD103+, possibly representing a discrete regulatory population.

Table 8.2.3 Phenotypes of T cells in nasal polyp suspensions

Cell type	**Marker**	**Percentage**[a]
Cytotoxic	CD3+ CD8+	20.2±5.4
Helper (by exclusion)	CD3+ CD8-	78.2±5.4
Mucosal	CD3+ CD103+	31.6±18.9
Conventional	CD3+ CD127+	41.3±11.5
Regulatory (natural)	CD3+ Foxp3+	2.8±0.5

[a]frequency within the T cell population

9 DISCUSSION

To date, there has been a conspicuous paucity of data in the literature regarding the production and regulation of allergen-induced inflammatory cytokines in human respiratory tissue.

This study represents, to the best of our knowledge, the first evidence of a critical functional role for T-cell mediated IL-10 in suppressing the allergen-specific production of IL-5, and to a lesser extent, IFN-γ, two signature cytokines associated with the inflammatory Th2 and Th1 pathways respectively, by *ex vivo* human respiratory mucosal cells derived from nasal polyps.

9.1 Summary of key findings

Nasal polyp cell suspensions were found to constitutively produce appreciable quantities of IL-10, IL-5 and IFN-γ, but not IL-2, IL-4 or TNF-α.

When stimulated with allergen, nasal polyp cell suspensions produced increased quantities of IL-10, but not IL-5, IFN-γ, or a range of other Th1 and Th2 cytokines (IL-2, IL-4 and TNF-α). IL-10 production was most significantly increased where the allergen used was the same as that the subject was sensitised to. When IL-10 was neutralised, there was a marked increase in IL-5 production, and to a lesser extent, IFN-γ and TNF-α production.

When T cells were depleted from cell suspensions, there was a marked reduction in IL-10 production in response to allergen, indicating that the observed IL-10 was largely derived from T cells or via a T cell dependent process.

Subsequent intracellular cytokine staining experiments demonstrated T cells immunoreactive for IL-10, but not IL-2 or IL-13, suggesting the observed IL-10 was derived or initiated from a regulatory T cell subset.

9.2 Constitutive production

Nasal polyp cell suspensions were found to constitutively produce appreciable quantities of IL-10, IL-5 and IFN-γ, but not IL-2, IL-4 or TNF-α.

Similar findings have previously been reported for nasal polyp T cell suspensions, with spontaneous production of IL-5 and IFN-γ, but no appreciable constitutive production of IL-2 and IL-4, by ELISA at 48 hours (343). Evidence of active *ex vivo* IL-5 and IFN-γ production whilst in suspension was demonstrated by the addition of cyclohexamide (a protein synthesis inhibitor), which resulted in complete ablation of measured cytokine production (343). Intracellular cytokine detection by ELISA at 8 hours, using Brefeldin A to accumulate intracellular cytokines, similarly demonstrated constitutive IL-5 and IFN-γ in NP T cells (344). RT-PCR of T cells derived from nasal polyps demonstrated IFN-γ production and, to a lesser extent, IL-10 (345) (IL-5 was not measured in this study).

Constitutive IL-5 production has been demonstrated to be markedly increased in NP tissue compared with Inferior turbinate (IT) tissue from non-polyposis controls (263, 362). IL-5 mRNA has been found in NP tissue but not in tissue from healthy controls (298, 363). IL-5 mRNA has been found to occur significantly more often in tissue from the anterior ethmoid (where most nasal polyps arise) compared to tissue from the lateral and medial surfaces of the middle turbinate (364).

Constitutive IFN-γ has been reported to be raised in NP compared with controls (345, 362). Other studies have found IFN-γ production to be not significantly different compared to controls (263). Some of the differences observed may relate to the atopic status of the test subjects.

Constitutive IL-5 and IFN-γ production has been demonstrated to be increased in T cells derived from nasal polyps compared to peripheral blood lymphocytes (343, 344).

Constitutive IL-10 production has been demonstrated to be present in tissue from patients with NP but decreased compared to controls (362).

We did not expect to see significant constitutive IL-2 or IL-4 production by CBA in nasal polyp cell suspensions as these cytokines are tightly regulated and rapidly internalised following expression.

The overall results for constitutive cytokine production confirmed the viability of our model.

9.3 Potential for cytokine production

To determine the functional capacity of T cells in nasal polyp cell suspensions to produce the cytokines under investigation, we added anti CD3/CD28 mAbs and found marked increases in production of IL-10, IL-5 and IFN-γ. Furthermore, IL-2, IL-4 and TNF-α, which were not constitutively detected in significant amounts, were all produced in measurable quantities with the addition of anti CD3/CD28 mAbs (Figure 7.5.1). This confirmed the presence of functioning T cells, capable of cytokine production, within our model.

Sanchez-Segura *et al* similarly added anti CD3/CD28 Abs to their NP T cell model to demonstrate a 5-fold and 60-fold increase in IL-5 and IFN-γ production, respectively (343). These results were of a similar order-of-magnitude to our own.

9.4 Significance of constitutive cytokines observed

The observed active constitutive production of IL-10, IL-5 and IFN-γ is suggestive of abnormal continuous T cell activation, resulting in a mixed Th1/ Th2 response.

NP T cells have been shown to express the T cell activation markers CD69, DR and CD54, whereas peripheral blood T cells from the same patients do not (343). These markers are associated with T cells in a special activation state typically associated with T cell accumulation at sites of chronic inflammation in conditions such as rheumatoid arthritis (365), inflammatory bowel disease (366), and Hashimoto's and Grave's thyroid diseases (367).

NP has been associated with *Staphylococcus aureus* (SA) colonisation, with NP patients demonstrated to have increased SA colonisation rates of the middle meatus, compared to controls (327). *Staphylococcus aureus* entertoxins (SAE) are expressed by SA and have superantigen activity. Superantigens have the capacity to cross link MHC class II on APCs and the TCR β-chain variable region on T cells, thereby activating T cells outside the conventional antigen-specific pathway (368). In this manner, up to 20-25% of T cells may be polyclonally activated (369). SAE-related polyclonal T cell activation in NP tissue results in Th2 and Th1 cytokine production, including IL-5 and IFN-γ (370). Such cytokine production, particularly IL-5, is thought to lead to eosinophilic inflammation and local production of IgE, including local polyclonal IgE production (370). This mechanism may explain the observed constitutive cytokine profile in some NP patients. However, not all NP patients demonstrate SA colonisation or polyclonal IgE production, suggesting that other mechanisms may play a role in other NP phenotypes.

Our results are consistent with a state of abnormal continuous T cell activation, resulting in a mixed Th1/ Th2 response.

9.5 Source of constitutive cytokines

9.5.1 IL-10

IL-10 is known to be produced particularly by Treg cells, but also by Th0, Th1, Th2 cells, B cells, DCs, macrophages, mast cells, monocytes and keratinocytes (187, 188).

CD4+CD25+ Treg cells have been demonstrated to anergize CD4+CD25- T cells by direct contact (359). Such anergized T cells may then be recruited to produce IL-10 and suppress CD4+ cells in an IL-10 dependent manner (359). This suggests that Treg cells initiate the production of IL-10, and then recruit bystander T cells to perform significant IL-10 synthesis.

In humans, the anti-inflammatory production of IL-10 has been studied most extensively in allergen specific immunotherapy (SIT). During successful SIT,

IL-10 has been demonstrated to initially increase intracellularly in the antigen-specific Treg cell population, with subsequent appearance in activated CD4+ T cells, monocytes and B cells (195). This suggests an autocrine/ paracrine action of Treg-cell-derived IL-10 as a key step in the induction of T-cell anergy and its subsequent maintenance by IL-10-producing APC and non-specifc bystander T cells (118).

Mast cells have recently been recognised as a potential source of IL-10 in some models of allergic contact dermatitis (360). This is thought to occur via antigen binding to receptors containing the FcR γ-chain. Whether this is a significant source in human airways is yet to be seen (371).

We hypothesised that the observed IL-10 was derived from T cells, following Treg initiation. Subsequent experiments provided support for this hypothesis (see *9.8 Source of up-regulated IL-10* and *9.9 T cell origin of observed IL-10*).

9.5.2 IL-5

In control nasal mucosa, there is little constitutive IL-5 production (264, 289, 323). By comparison, in NP, IL-5 is uniformly elevated in atopics and non-atopics (275, 298, 323).

Whilst T cells, particularly Th2 cells, are recognised as a primary source of IL-5, in chronic eosinophillic inflammation, eosinophils may contribute, or even provide the dominant source (170). In NP, most tissue eosinophils are in an activated state (372). In a study of NP T cell phenotypes and intracellular cytokine production, it was found that IL-4 was the predominant Th2 cytokine found intracellularly in T cells (344). However, IL-5 is consistently measured in greater quantities in NP tissue (rather than intracellularly in T cells alone) (258, 275, 298, 372), suggesting a non-T cell source of IL-5. In NP, the principal source of IL-5 appears to be eosinophils (258, 298, 343, 344).

9.5.3 IFN-γ

There are significantly more IFN-γ producing T cells in NP tissue than peripheral blood (344). Furthermore, the proportion of both CD4+ and CD8+ T

cells in NP tissue producing IFN-γ is significantly greater than the population producing IL-5 (344). In NP, the principal source of IFN-γ appears to be T cells.

The observations that IFN-γ producing T cells outnumber those producing IL-5, and that IFN-γ is principally produced by T cells, whereas IL-5 is principally produced by eosinophils, is consistent with our findings that IL-5 is constitutively produced in greater quantities from NP cell suspensions than IFN-γ, but that T cell stimulation with anti CD3/CD28 mAbs results in markedly greater quantities of IFN-γ.

This view is supported by the finding that RT-PCR of T cells derived from nasal polyps results in significantly greater IFN-γ than IL-10 (345).

9.6 Cytokine response to added allergen

When stimulated with allergen, nasal polyp cell suspensions did not produce increased quantities of the signature Th2 (IL-5, IL-4) or Th1 (IFN-γ, IL-2, TNF-α) cytokines. In fact, IFN-γ demonstrated a non-significant trend towards *decreased* production (Figure 5.5.5). Furthermore, subgroup analysis of atopics and non-atopics failed to show significant variation in cytokine production, although there was a non-significant trend towards increased IL-5 production in atopics.

These results suggest a regulatory mechanism is in place, limiting the inflammatory response to allergen stimulation in NP.

Furthermore, when stimulated with allergen, nasal polyp cell suspensions produced increased quantities of IL-10 in a dose-response fashion (Figure 3.7.1). The effect was most significant in cells derived from atopic subjects, where the subject was sensitised to the added allergen (Figure 3.7.2).

These results suggest the presence of a population of allergen-specific cells within nasal polyp tissue that respond to allergen stimulation with the production of IL-10. Cell suspensions from atopic subjects are most likely to contain cell populations that are skewed towards the specific allergen to which

they are sensitised, and reduced numbers of cells specific to non-sensitised allergens. This is borne out by the observed attenuated IL-10 response in atopics to non-sensitised allergen (Figure 3.7.2).

9.7 IL-10 regulation of Th1 and Th2 cytokines

IL-10 is recognised as the most important anti-inflammatory cytokine in humans (373). IL-10 is a potent inhibitor of both Th1 cytokine responses including IFN-γ, IL-2 and TNF-α (189) and Th2 cytokine responses, including IL-5 and IL-4 (192-194). IL-10 specifically down-regulates the production of these cytokines by T cells (192-194). It also reduces the release of pro-inflammatory cytokines by mast cells (194) and eosinophils (199, 200), amongst numerous other functions (see *1.4.3.2 Interleukin 10*).

We hypothesised that the allergen-specific IL-10 response observed was inhibiting the allergen-specific production of Th1 and Th2 cytokines. To test this hypothesis, we added anti-IL-10 antibodies to polyp cell suspensions and measured the cytokine response to added allergen. Neutralisation of IL-10 resulted in a marked increase in IL-5 production, along with a less significant increase in IFN-γ and TNF-α, in response to added allergen.

This suggests an IL-10 dependent regulatory mechanism that normally suppresses abnormal Th2, and to a lesser extent, Th1, responses to allergen in nasal polyps.

This confirms recent *in vivo* murine experiments demonstrating the critical functional role of CD4+CD25+ T cell derived IL-10 in suppressing allergic responses within the airway (127-129).

9.8 Source of up-regulated IL-10

To further clarify the source of the observed IL-10 we depleted T cells from nasal polyp cell suspensions and allergen-specific cytokine production was determined. T cell depletion significantly reduced IL-10 production in response to allergen, compared to a column control, which was unchanged. This

indicated that the observed IL-10 was derived either from T cells or via a T cell dependent process.

This supported our hypothesis that the allergen-specific IL-10 observed was produced in T cells following Treg cell initiation.

Whilst mast cells have recently been recognised as a potential source of IL-10 in some models of allergic contact dermatitis (360), this mechanism is largely T-cell independent and therefore, unlikely to be a major source in the nasal polyp cell suspensions studied in our experiments.

9.9 T cell origin of observed IL-10

The non-T cell sources of IL-10 (B cells, DCs, macrophages, mast cells, monocytes and keratinocytes) still largely require T cell derived cytokines for priming, survival and activity (374, 375). It was therefore necessary to demonstrate specific T cell production of the observed IL-10. This was achieved through intracellular cytokine staining. T cells immunoreactive for IL-10, but not IL-2 or IL-13 were found, suggesting the observed IL-10 was derived from a non-Th1 and non-Th2 T cell subset – most likely regulatory T cells or T cells anergized by Tregs (359).

9.10 T cells in nasal polyposis

Studies of nasal polyp T cell subsets have found that most NP T cells express CD45RO, indicating they are mostly memory T cells (343, 345). NP T cells express significantly less TCR, CD3 and CD28, compared to peripheral blood T cells, similarly indicating that NP T cells are largely mature memory T cells, requiring less antigen-recognition signals and minimal co-stimulation for activation to occur (343).

Other groups have similarly demonstrated differences between T cells derived from NP cell suspensions and peripheral blood. Bernstein *et al* found NP cell suspensions had a significantly higher proportion of CD8+ vs CD4+ T cells than peripheral blood (344). Other groups have found NP cell suspensions

contain significantly more CD8+ and CD4+ cells than mucosal tissue from controls (345, 362).

The adhesion molecule CD103 has been identified as a mucosal homing marker on Treg cells, distinguishing Tregs that migrate to mucosal sites of inflammation (CD103+) from those that recirculate through the lymphoid tissues (376). Our experiments demonstrated a significant minority of CD103+ T cells (Table 8.2.3). NP T cells express the adhesion molecule CD103 in significantly greater quantities than peripheral blood T cells, indicating a subset of NP T cells intended for retention within the nasal mucosa (343).

Despite demonstrating significant numbers of CD103+ T cells, our experiments showed relatively low numbers of Foxp3+ T cells. In contrast to the mouse, human CD103+ T cells rarely express Foxp3 (377), implying that at least some of these cells may represent a discrete subset of Treg cells that are Foxp3-CD103+.

Foxp3 expression has been shown to be reduced in nasal tissue from subjects with CRSwNP compared to CRSsNP and controls (362, 378). Van Bruaene *et al* concluded that NP may be a consequence of defective Treg suppressive function (378). However, in the same study, IL-10 levels by mRNA, were shown to be similar in nasal tissue from subjects with CRSwNP and CRSsNP. This may instead suggest that, in NP, a subset of IL-10 producing, inducible Foxp3- Tregs my play a more significant suppressive role than thymic-derived natural Foxp3+ Tregs.

By contrast, Li *et al* found reduced Foxp3 *and* IL-10 in nasal polyps compared with control mucosa (379). Subjects were then treated with intranasal steroids (mometasone 50mcg/day) for four weeks. Post-treatment biopsies demonstrated increased Foxp3 and IL-10, suggesting that intranasal steroids suppress inflammation via an increase in Foxp3+ Treg numbers and/or function.

CD4+CD25+Foxp3+ Treg cells have been found to be increased in NP patients with eosinophil-predominant disease compared to neutrophil-predominant NP patients (380)

9.11 Treg/ Th17 balance

Shen *et al* investigated the balance between Treg and Th17 cells in NP in the tissues and peripheral blood. They found a significant imbalance towards Th17 vs Treg in NP in both tissues and blood, that was more marked in atopics compared to non-atopics (262). Similar Th17/ Treg imbalances have been observed in other chronic inflammatory diseases, including inflammatory bowel disease and juvenile arthritis (381, 382). Treg cell presence was found to be negatively correlated with Th1 and Th2 cytokines. However, Th17 cells were not found to correlate with Th1 and Th2 cytokines (262). The significance of these findings to our results is uncertain, particularly as they specifically refer to a South-east Asian population, where neutrophil-predominant NP is more common that the eosinophil-predominant NP population of Central London, where our patients were recruited.

9.12 IgE

It is well established that tissue IgE levels are raised in NP compared to controls, particularly in atopics (258, 323, 362). In atopics with NP there is a strong correlation between tissue and serum IgE. In non-atopics with NP, tissue IgE is often raised but the correlation with serum IgE is poor (323).

9.13 Polyclonal IgE

Staphylococcus aureus enterotoxins (SAE) have been shown to play an important role in NP and asthma (276). In a significant subset of NP patients, SAE act as a "superantigen", polyclonally activating T-cells, resulting in polyclonal IgE production, high total tissue IgE titres, increased eosinophilic inflammation, high ECP concentrations, increased Th2 cytokines, particularly IL-5, and Treg inhibition (383).

There has been some debate over whether this polyclonal IgE is functional (384). Recent evidence has demonstrated that specific IgE to grass pollen and other allergens within the polyclonal IgE found in NP tissues is functional (383).

In AR patients, inferior turbinate (IT) tissue IgE and serum IgE are highly correlated (383). By contrast, tissue IgE in NP patients is markedly elevated compared to serum. In NP patients, mast cell degranulation in response to specific allergen correlates with the presence of specific IgE to that allergen in the polyclonal IgE within polyp tissue (383). However, specific IgE to that particular allergen is often not found in the serum of the same patient (383). This capacity of NP polyclonal IgE to react to multiple inhalant allergens may act to continuously stimulate mast cells to release immune mediators. However, due to the polyclonal nature of NP IgE, mast cell activation and mediator release may tend to be in lower quantities, compared with AR, leading to continuous low grade chronic inflammation rather than acute attacks (383).

These findings would be consistent with our observed constitutive Th1/ Th2 cytokine production and potential for allergen-specific cytokine response, following neutralisation of IL-10.

9.14 IgA

IgA has been demonstrated to be the predominant immunoglobulin present constitutively and actively produced in NP tissue *in vitro* (310), compared to peripheral blood, where IgG predominates (385). Blockade of IL-10 and TNF-α was found to reduce IgA secretion by around 50%, indicating both cytokines are critical for nasal polyp plasma cell terminal differentiation (385). Interestingly, this study failed to show significant IgE presence or production, except in one patient with AR. Unfortunately, all other patients studied were non-atopic, limiting the applicability of the results regarding IgE. However, these findings do confirm that NP includes several phenotypes, of which not all require a significant role for IgE.

9.15 Mast cells

Interestingly, investigations into the effect of SAE in NP have demonstrated that, whereas *S aureus* enterotxin B had no effect on mast cells, another antigen, surface protein A (SpA), resulted in mast cell cytokine release (370).

Mast cells are recognised as inflammatory effector cells of the immune system at mucosal surfaces. However, it has recently been recognised that mast cells have an additional function in the induction and regulation of the immune response (386). Mast cells may influence the T cell response towards Th1, Th2 or Th17 (387, 388) and B cell survival, proliferation and IgA production (389).

In addition, recent evidence has come to light demonstrating that, in chronic inflammation, mast cells have the capacity to induce IL-10 producing Tregs (390). The evidence regarding mast cells and inflammatory regulation in NP is yet to be established. Our results indicate immune co-operation may occur with mast cells, but that mast cells are not the main source of the cytokines observed.

9.16 Potential limitations of the work and future directions

In critically self-appraising the methods and data presented, we have identified several potential areas of limitation of the work, along with several avenues for future investigation. The following is a discussion around these areas:

9.16.1 Method of determining atopy

For the purposes of this study, we chose to use skin prick testing as the primary method for determining atopic status. We chose not to use peripheral blood IgE as we felt that the specific *in vivo* test of clinical responsiveness (skin prick testing) is more sensitive than the *in vitro* test of immunological response (serum specific IgE), and therefore more relevant to the interpretation of data in this study.

9.16.2 Non-responders

5 out of 30 experiments failed to respond with significant cytokine production, either constitutively or following allergen stimulation (see Appendix 2 – raw data). It is unclear why this occurred. There was no evidence of infection and

atopic status was not a significant factor as there were 3 atopics and 2 non-atopics within the non-responder group.

9.16.3 Local IgE production

“Entopy” refers to the mucosal production of specific IgE in the absence of serum IgE and with negative skin prick testing (391). This concept remains somewhat controversial, but has been proposed as a possible aetiological factor in up to 40% of “non-allergic” rhinitis and has been identified in the bronchial tissues of both non-atopic and atopic asthmatics (392). Unfortunately, useful clinical tests of entopy (nasal challenge testing) are not widely available, but could certainly be helpful in further dissecting the data in our and similar studies. In particular, the presence of polyclonal IgE in around 50% of Western NP patients (276) makes the issue of entopy relevant to this discussion.

9.16.4 Cat and grass allergen vs HDM allergen

The potentially contrasting IL-10 responses seen with HDM allergen, compared with cat and grass allergens were intriguing, but the study group in that subset was not sufficiently powered to dissect the data to that level. It would be interesting to study this specific area further.

9.16.5 Nasal polyposis phenotypes

The fact that several NP phenotypes exist, including eosinophil-predominant and neutrophil-predominant, along with multiple co-morbidities, including aspirin-sensitivity, cystic fibrosis, Churg-Strauss disease and others, introduces variables that could distort our data. Ideally, such subgroups would be studied independently. However, our supply of experimental tissue was determined by the clinical workload of the hospital and, as such, we were obliged to process material without regard to phenotype. Sub-group analysis of phenotype and co-morbidity would require a significantly greater number of subjects.

9.16.6 Polyp versus non-polypoid mucosa

It would be very interesting to continue this line of enquiry with a series of experiments assessing allergen-specific cytokine production using inferior turbinate tissue in patients with and without AR. In particular, to compare the results using both polyp and inferior turbinate tissue harvested from patients with both AR and NP. Inferior turbinate tissue is often removed during sino-nasal surgery and therefore potentially available for research purposes. One of the difficulties we encountered is that the yield of cells from small quantities of inferior turbinate tissue does not compare to the large number of cells that may be isolated from large nasal polyps.

Evidence is emerging that the inflammatory processes vary significantly between different sino-nasal sites, including nasal polyps, the inferior turbinate and the middle turbinate (240). Unfortunately, middle turbinate tissue is much less frequently removed surgically, making availability of this material for research purposes significantly more difficult.

9.16.7 Non-polyposis controls

Similarly, it would have been very interesting to study the sino-nasal mucosa of non-polyposis control subjects. However, ethical concerns and the volume of tissue required limited this potential area of study.

10 CONCLUSIONS

Allergen-specific regulatory T cells play a critical functional role in human respiratory tissue derived from nasal polyps by regulating abnormal Th2 and Th1 responses to common inhaled aeroallergens, through mechanisms dependent on allergen-specific production of IL-10.

REFERENCES

1. Bousquet J, Van Cauwenberge P, Khaltaev N. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol. 2001 Nov; 108(5 Suppl):S147-334.
2. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy. 2008 Apr; 63 Suppl 86:8-160.
3. Taylor WR, Newacheck PW. Impact of childhood asthma on health. Pediatrics. 1992 Nov; 90(5):657-62.
4. Nathan RA, Meltzer EO, Selner JC, Storms W. The prevalence and medical and economic impact of allergic rhinitis in the United States. J Allergy Clin Immunol. 1997 Jun; 99(6 Pt 2):S808-14.
5. Wright AL, Holberg CJ, Martinez FD, Halonen M, Morgan W, Taussig LM. Epidemiology of physician-diagnosed allergic rhinitis in childhood. Pediatrics. 1994 Dec; 94(6 Pt 1):895-901.
6. Galassi C, De Sario M, Biggeri A, Bisanti L, Chellini E, Ciccone G, et al. Changes in Prevalence of Asthma and Allergies Among Children and Adolescents in Italy: 1994-2002. Pediatrics. 2006 January 1, 2006; 117(1):34-42.
7. Asher MI, Montefort S, Bjorksten B, Lai CKW, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. The Lancet. 2006; 368(9537):733-43.

8. McMenamin P. Costs of hay fever in the United States in 1990. Ann Allergy. 1994 Jul; 73(1):35-9.

9. Gregory C, Cifaldi M, Tanner LA. Targeted intervention programs: creating a customized practice model to improve the treatment of allergic rhinitis in a managed care population. Am J Manag Care. 1999 Apr; 5(4):485-96.

10. Mosges R, Klimek L. Today's allergic rhinitis patients are different: new factors that may play a role. Allergy Theme: Highlights in allergic and non-allergic rhinitis. 2007; 62(9):969-75.

11. van Aas K, Aberg N, Bachert C. Socio-economic costs of allergic diseases: epidemiology- prevalence of allergic diseases. European Allergy White Paper. Brussels: The UCB Institute of Allergy; 1997.

12. Kaiser HB. Risk factors in allergy/asthma. Allergy Asthma Proc. 2004 Jan-Feb; 25(1):7-10.

13. Liu AH. Endotoxin exposure in allergy and asthma: reconciling a paradox. J Allergy Clin Immunol. 2002 Mar; 109(3):379-92.

14. Faith AP, McDonald JM, Peek EM, Richards DM, Caulfield JM, Chevretton EMD, et al. Functional plasticity of human respiratory tract dendritic cells: GM-CSF enhances TH2 development. Journal of Allergy & Clinical Immunology. 2005; 116(5):1136-43.

15. Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, Toll-like Receptor 4-dependent T Helper Cell Type 2 Responses to Inhaled Antigen. J Exp Med. 2002 December 16, 2002; 196(12):1645-51.

16. Nigo YI, Yamashita M, Hirahara K, Shinnakasu R, Inami M, Kimura M, et al. Regulation of allergic airway inflammation through Toll-like receptor 4-mediated modification of mast cell function. Proceedings of the National Academy of Sciences of the United States of America. 2006 Feb 14; 103(7):2286-91.

17. Hammad H, Charbonnier A-S, Duez C, Jacquet A, Stewart GA, Tonnel A-B, et al. Th2 polarization by Der p 1-pulsed monocyte-derived dendritic cells is due to the allergic status of the donors. Blood. 2001 August 15, 2001; 98(4):1135-41.

18. Hammad H, Smits HH, Ratajczak C, Nithiananthan A, Wierenga EA, Stewart GA, et al. Monocyte-derived dendritic cells exposed to Der p 1 allergen enhance the recruitment of Th2 cells: major involvement of the chemokines TARC/CCL17 and MDC/CCL22. Eur Cytokine Netw. 2003 Oct-Dec; 14(4):219-28.

19. Foley S, Hamid Q. Inflammatory patterns in allergic rhinitis. Clinical & Experimental Allergy Reviews. 2006; 6(4):91-5.

20. Lebel B, Bousquet J, Morel A, Chanal I, Godard P, Michel FB. Correlation between symptoms and the threshold for release of mediators in nasal secretions during nasal challenge with grass-pollen grains. J Allergy Clin Immunol. 1988 Nov; 82(5 Pt 1):869-77.

21. Bradding P, Feather IH, Howarth PH, Mueller R, Roberts JA, Britten K, et al. Interleukin 4 is localized to and released by human mast cells. J Exp Med. 1992 Nov 1; 176(5):1381-6.

22. Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, et al. Interleukin-4, -5, and -6 and tumor necrosis factor-

alpha in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. Am J Respir Cell Mol Biol. 1994 May; 10(5):471-80.

23. Bradding P. Human mast cell cytokines. Clinical and Experimental Allergy : Journal of the British Society for Allergy and Clinical Immunology. 1996 Jan; 26(1):13-9.

24. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF, Jr., Meyers DA, Norman PS, et al. Mediator release after nasal airway challenge with allergen. Am Rev Respir Dis. 1983 Oct; 128(4):597-602.

25. White M. Mediators of inflammation and the inflammatory process. J Allergy Clin Immunol. 1999 Mar; 103(3 Pt 2):S378-81.

26. Dunnette SL, Kita H, Reed CE. Topical glucocorticoid treatment of ragweed hay fever. II. Relationship of eosinophil-derived neurotoxin (EDN) and IL-5 to disease severity. J Allergy Clin Immunol. 1997; 99:124-8.

27. Roche N, Chinet TC, Huchon GJ. Allergic and nonallergic interactions between house dust mite allergens and airway mucosa. The European Respiratory Journal : Official Journal of the European Society for Clinical Respiratory Physiology. 1997 Mar; 10(3):719-26.

28. Thompson PJ. Unique role of allergens and the epithelium in asthma. Clinical and Experimental Allergy : Journal of the British Society for Allergy and Clinical Immunology. 1998 Nov; 28 Suppl 5:110-6; discussion 7-8.

29. King CL, Malhotra I, Mungai P, Wamachi A, Kioko J, Ouma JH, et al. B cell sensitization to helminthic infection develops in utero in humans. Journal of Immunology. 1998 Apr 1; 160(7):3578-84.

30. Jacquet A. The role of innate immunity activation in house dust mite allergy. Trends Mol Med. 2011 Oct; 17(10):604-11.

31. Bauchau V, Durham SR. Epidemiological characterization of the intermittent and persistent types of allergic rhinitis. Allergy. 2005; 60(3):350-3.

32. Bousquet J, Annesi-Maesano I, Carat F, Leger D, Rugina M, Pribil C, et al. Characteristics of intermittent and persistent allergic rhinitis: DREAMS study group. Clinical & Experimental Allergy. 2005; 35(6):728-32.

33. Valovirta Ea, Myrseth S-Eb, Palkonen Sb. The voice of the patients: allergic rhinitis is not a trivial disease. Current Opinion in Allergy & Clinical Immunology. 2008; 8(1):1-9.

34. Van Hoecke H, Vastesaeger N, Dewulf L, Sys L, van Cauwenberge P. Classification and management of allergic rhinitis patients in general practice during pollen season. Allergy Theme: Upper and lower airway link confirmed. 2006; 61(6):705-11.

35. Thompson AK, Juniper E, Meltzer EO. Quality of life in patients with allergic rhinitis. Ann Allergy Asthma Immunol. 2000 Nov; 85(5):338-47; quiz 47-8.

36. Reilly MC, Tanner A, Meltzer EO. Work, Classroom and Activity Impairment Instruments: Validation Studies in Allergic Rhinitis. Clinical Drug Investigation. 1996; 11(5):278-88.

37. Meltzer EO, Nathan RA, Seiner JC, Storms W. Quality of life and rhinitic symptoms: Results of a nationwide survey with the SF-36 and RQLQ questionnaires. J Allergy Clin Immunol. 1997; 99(6):S815-S9.

38. Vuurman EF, van Veggel LM, Uiterwijk MM, Leutner D, O'Hanlon JF. Seasonal allergic rhinitis and antihistamine effects on children's learning. Ann Allergy. 1993 Aug; 71(2):121-6.

39. Settipane RA. Complications of Allergic Rhinitis. Allergy and Asthma Proceedings. 1999; 20:209-13.

40. Spector SL. Overview of comorbid associations of allergic rhinitis. J Allergy Clin Immunol. 1997 Feb; 99(2):S773-80.

41. Slavin RG. Sinusitis in adults and its relation to allergic rhinitis, asthma, and nasal polyps. J Allergy Clin Immunol. 1988 Nov; 82(5 Pt 2):950-6.

42. Corey JP, Adham RE, Abbass AH, Seligman I. The role of IgE-mediated hypersensitivity in otitis media with effusion. Am J Otolaryngol. 1994 Mar-Apr; 15(2):138-44.

43. Smart B. The costs of asthma and allergy. American Academy of Allergy, Asthma, and Immunology; [cited 2005 19/05]; Available from: http://www.aaaai.org/patients/advocate/2004/fall/costs.

44. Kaliner MA. Nonallergic rhinopathy (formerly known as vasomotor rhinitis). Immunol Allergy Clin North Am. 2011 Aug; 31(3):441-55.

45. Sin B, Togias A. Pathophysiology of allergic and nonallergic rhinitis. Proc Am Thorac Soc. 2011 Mar; 8(1):106-14.

46. Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention 2006. Available from: www.ginasthma.org.

47. National Asthma Council Australia. Asthma managment handbook. South Melbourne 2006.

48. Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy. 2004 May; 59(5):469-78.

49. Guerra S, Sherrill DL, Martinez FD, Barbee RA. Rhinitis as an independent risk factor for adult-onset asthma. J Allergy Clin Immunol. 2002 Mar; 109(3):419-25.

50. Castro-Rodriguez JA, Holberg CJ, Wright AL, Halonen M, Taussig LM, Morgan WJ, et al. Association of radiologically ascertained pneumonia before age 3 yr with asthmalike symptoms and pulmonary function during childhood: a prospective study. Am J Respir Crit Care Med. 1999 Jun; 159(6):1891-7.

51. Eisner MD, Yelin EH, Henke J, Shiboski SC, Blanc PD. Environmental tobacco smoke and adult asthma. The impact of changing exposure status on health outcomes. Am J Respir Crit Care Med. 1998 Jul; 158(1):170-5.

52. Ford ES. The epidemiology of obesity and asthma. J Allergy Clin Immunol. 2005 May; 115(5):897-909; quiz 10.

53. National Heart Lung and Blood Institute - Expert Panel Report 2. Guidelines for the Diagnosis and Management of Asthma. Bethesda, MD: National Institutes of Health pub no. 97-4051; 1997.

54. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. N Engl J Med. 2002 May 30; 346(22):1699-705.

55. Green RH, Brightling CE, Bradding P. The reclassification of asthma based on subphenotypes. Curr Opin Allergy Clin Immunol. 2007 Feb; 7(1):43-50.

56. Barnett SB, Nurmagambetov TA. Costs of asthma in the United States: 2002-2007. J Allergy Clin Immunol. 2011 Jan; 127(1):145-52.

57. Mannino DM, Homa DM, Akinbami LJ, Moorman JE, Gwynn C, Redd SC. Surveillance for asthma--United States, 1980-1999. MMWR Surveill Summ. 2002 Mar 29; 51(1):1-13.

58. Passalacqua G, Ciprandi G, Canonica GW. United airways disease: therapeutic aspects. Thorax. 2000 Oct; 55 Suppl 2:S26-7.

59. Corren J. Allergic rhinitis and asthma: how important is the link? J Allergy Clin Immunol. 1997 Feb; 99(2):S781-6.

60. Lombardi C, Passalacqua G, Gargioni S, Senna G, Ciprandi G, Scordamaglia A, et al. The natural history of respiratory allergy: a follow-up study of 99 patients up to 10 years. Respir Med. 2001 Jan; 95(1):9-12.

61. Settipane RJ, Hagy GW, Settipane GA. Long-term risk factors for developing asthma and allergic rhinitis: a 23-year follow-up study of college students. Allergy Proc. 1994 Jan-Feb; 15(1):21-5.

62. Greisner WA, 3rd, Settipane RJ, Settipane GA. Co-existence of asthma and allergic rhinitis: a 23-year follow-up study of college students. Allergy Asthma Proc. 1998 Jul-Aug; 19(4):185-8.

63. Togias A. Rhinitis and asthma: evidence for respiratory system integration. J Allergy Clin Immunol. 2003 Jun; 111(6):1171-83; quiz 84.

64. European Community Respiratory Health Survey II Steering Committee. The European Community Respiratory Health Survey II. Eur Respir J. 2002 Nov; 20(5):1071-9.

65. Linneberg A, Henrik Nielsen N, Frolund L, Madsen F, Dirksen A, Jorgensen T. The link between allergic rhinitis and allergic asthma: a prospective population-based study. The Copenhagen Allergy Study. Allergy. 2002 Nov; 57(11):1048-52.

66. Simons FE. Allergic rhinobronchitis: the asthma-allergic rhinitis link. J Allergy Clin Immunol. 1999 Sep; 104(3 Pt 1):534-40.

67. Canbaz P, Uskudar-Teke H, Aksu K, Keren M, Gulbas Z, Kurt E. Nasal eosinophilia can predict bronchial hyperresponsiveness in persistent rhinitis: evidence for united airways disease concept. American Journal of Rhinology & Allergy. 2011 Mar-Apr; 25(2):120-4.

68. Moller C, Dreborg S, Ferdousi HA, Halken S, Host A, Jacobsen L, et al. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). J Allergy Clin Immunol. 2002 Feb; 109(2):251-6.

69. Pedersen W, Hjuler I, Bisgaard H, Mygind N. Nasal inhalation of budesonide from a spacer in children with perennial rhinitis and asthma. Allergy. 1998 Apr; 53(4):383-7.

70. Camargos PA, Rodrigues ME, Lasmar LM. Simultaneous treatment of asthma and allergic rhinitis. Pediatr Pulmonol. 2004 Sep; 38(3):186-92.

71. Camargos P, Ibiapina C, Lasmar L, Cruz AA. Obtaining concomitant control of allergic rhinitis and asthma with a nasally inhaled corticosteroid. Allergy. 2007 Mar; 62(3):310-6.

72. Kersten ET, van Leeuwen JC, Brand PL, Duiverman EJ, de Jongh FH, Thio BJ, et al. Effect of an intranasal corticosteroid on exercise induced bronchoconstriction in asthmatic children. Pediatr Pulmonol. 2012 Jan; 47(1):27-35.

73. Brannan JD, Turton JA. The inflammatory basis of exercise-induced bronchoconstriction. Phys Sportsmed. 2010 Dec; 38(4):67-73.

74. Greisner WA, 3rd, Settipane RJ, Settipane GA. Natural history of hay fever: a 23-year follow-up of college students. Allergy Asthma Proc. 1998 Sep-Oct; 19(5):271-5.

75. Greisner WA, 3rd, Settipane RJ, Settipane GA. The course of asthma parallels that of allergic rhinitis: a 23-year follow-up study of college students. Allergy Asthma Proc. 2000 Nov-Dec; 21(6):371-5.

76. Stelmach R, do Patrocinio TNM, Ribeiro M, Cukier A. Effect of treating allergic rhinitis with corticosteroids in patients with mild-to-moderate persistent asthma. Chest. 2005 Nov; 128(5):3140-7.

77. Watson WT, Becker AB, Simons FE. Treatment of allergic rhinitis with intranasal corticosteroids in patients with mild asthma: effect on lower airway responsiveness. J Allergy Clin Immunol. 1993 Jan; 91(1 Pt 1):97-101.

78. Fuhlbrigge AL, Adams RJ. The effect of treatment of allergic rhinitis on asthma morbidity, including emergency department visits. Curr Opin Allergy Clin Immunol. 2003 Feb; 3(1):29-32.

79. Reed CE, Marcoux JP, Welsh PW. Effects of topical nasal treatment on asthma symptoms. J Allergy Clin Immunol. 1988 May; 81(5 Pt 2):1042-7.

80. Henriksen JM, Wenzel A. Effect of an intranasally administered corticosteroid (budesonide) on nasal obstruction, mouth breathing, and asthma. Am Rev Respir Dis. 1984 Dec; 130(6):1014-8.

81. Corren J, Adinoff AD, Buchmeier AD, Irvin CG. Nasal beclomethasone prevents the seasonal increase in bronchial responsiveness in patients with allergic rhinitis and asthma. J Allergy Clin Immunol. 1992 Aug; 90(2):250-6.

82. Wood RA, Eggleston PA. The effects of intranasal steroids on nasal and pulmonary responses to cat exposure. Am J Respir Crit Care Med. 1995 Feb; 151(2 Pt 1):315-20.

83. Pawankar R. Allergic rhinitis and asthma: are they manifestations of one syndrome? Clin Exp Allergy. 2006 Jan; 36(1):1-4.

84. Marple BFMD. The Unified Airway: Do We Fully Appreciate Its Impact in Otolaryngology? ENToday. 2007; 2(8):3-4.

85. Lipworth BJ, White PS. Allergic inflammation in the unified airway: start with the nose. Thorax. 2000 October 1, 2000; 55(10):878-81.

86. Noelle R, Snow EC. T helper cells. Current Opinion in Immunology. 1992 Jun; 4(3):333-7.

87. Prioult G, Nagler-Anderson C. Mucosal immunity and allergic responses: lack of regulation and/or lack of microbial stimulation? Immunological Reviews. 2005; 206(1):204-18.

88. Coffman RL. Origins of the T(H)1-T(H)2 model: a personal perspective. Nature Immunology. 2006 Jun; 7(6):539-41.

89. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology. 1989; 7:145-73.

90. Pasare C, Medzhitov R. Toll-like receptors: linking innate and adaptive immunity. Adv Exp Med Biol. 2005; 560:11-8.

91. Wang YH, Ito T, Homey B, Watanabe N, Martin R, Barnes CJ, et al. Maintenance and polarization of human TH2 central memory T cells by thymic stromal lymphopoietin-activated dendritic cells. Immunity. 2006 Jun; 24(6):827-38.

92. Taylor APab, Akdis MMDPa, Joss APa, Akkoc TPac, Wenig RPd, Colonna MMDe, et al. IL-10 inhibits CD28 and ICOS costimulations of T cells via src homology 2 domain-containing protein tyrosine phosphatase 1. Journal of Allergy & Clinical Immunology. 2007; 120(1):76-83.

93. Baxter AG, Hodgkin PD. Activation rules: the two-signal theories of immune activation. Nature Reviews Immunology. 2002 Jun; 2(6):439-46.

94. Croft M. Costimulation of T cells by OX40, 4-1BB, and CD27. Cytokine Growth Factor Rev. 2003 2003 Jun-Aug; 14(3-4):265-73.

95. Tarkowski M. Expression and a role of CD30 in regulation of T-cell activity. Curr Opin Hematol. 2003 Jul; 10(4):267-71.

96. Grimbacher B, Warnatz K, Peter HH. The immunological synapse for B-cell memory: the role of the ICOS and its ligand for the longevity of humoral immunity. Curr Opin Allergy Clin Immunol. 2003 Dec; 3(6):409-19.

97. Jaffar ZH, Stanciu L, Pandit A, Lordan J, Holgate ST, Roberts K. Essential role for both CD80 and CD86 costimulation, but not CD40 interactions, in allergen-induced Th2 cytokine production from asthmatic bronchial tissue: role for alphabeta, but not gammadelta, T cells. J Immunol. 1999 Dec 1; 163(11):6283-91.

98. Sheridan BS, Lefrancois L. Regional and mucosal memory T cells. Nature Immunology. 2011 Jun; 12(6):485-91.

99. Strachan DP. Family size, infection and atopy: the first decade of the "hygiene hypothesis". Thorax. 2000 Aug; 55 Suppl 1:S2-10.

100. Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med. 2002 Sep 19; 347(12):911-20.

101. Sheikh A, Smeeth L, Hubbard R. There is no evidence of an inverse relationship between TH2-mediated atopy and TH1-mediated autoimmune disorders: Lack of support for the hygiene hypothesis. J Allergy Clin Immunol. 2003 Jan; 111(1):131-5.

102. Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. Science. 2002 Apr 19; 296(5567):490-4.

103. Bashir ME, Andersen P, Fuss IJ, Shi HN, Nagler-Anderson C. An enteric helminth infection protects against an allergic response to dietary antigen. J Immunol. 2002 Sep 15; 169(6):3284-92.

104. Hansen G, Berry G, DeKruyff RH, Umetsu DT. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperreactivity but cause severe airway inflammation. J Clin Invest. 1999 Jan; 103(2):175-83.

105. Wills-Karp M, Santeliz J, Karp CL. The germless theory of allergic disease: revisiting the hygiene hypothesis. Nat Rev Immunol. 2001 Oct; 1(1):69-75.

106. Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J, et al. Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. Lancet. 2004 Feb; 363(9409):608-15.

107. Ito T, Hanabuchi S, Wang YH, Park WR, Arima K, Bover L, et al. Two functional subsets of FOXP3+ regulatory T cells in human thymus and periphery. Immunity. 2008 Jun; 28(6):870-80.

108. Hawrylowicz CM, O'Garra A. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. Nature Reviews Immunology. 2005 Apr; 5(4):271-83.

109. Roncarolo MG, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K, Levings MK. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. Immunological Reviews. 2006 Aug; 212:28-50.

110. Hawrylowicz CM. Regulatory T cells and IL-10 in allergic inflammation. J Exp Med. 2005 Dec 5; 202(11):1459-63.

111. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol. 2003 Apr; 4(4):330-6.

112. Carter L, Fouser LA, Jussif J, Fitz L, Deng B, Wood CR, et al. PD-1:PD-L inhibitory pathway affects both CD4(+) and CD8(+) T cells and is overcome by IL-2. Eur J Immunol. 2002 Mar; 32(3):634-43.

113. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity. 1999 Aug; 11(2):141-51.

114. Jutel M, Akdis CA. T-cell subset regulation in atopy. Current Allergy and Asthma Reports. 2011 Apr; 11(2):139-45.

115. Taylor A, Verhagen J, Blaser K, Akdis M, Akdis CA. Mechanisms of immune suppression by interleukin-10 and transforming growth factor-[beta]: the role of T regulatory cells. Immunology. 2006; 117(4):433-42.

116. Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Crameri R, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. J Exp Med. 2004 Jun 7; 199(11):1567-75.

117. Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, et al. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. The Journal of Clinical Investigation. 2000 Dec; 106(12):R75-81.

118. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. J Clin Invest. 1998 Jul 1; 102(1):98-106.

119. Karagiannidis C, Akdis M, Holopainen P, Woolley NJ, Hense G, Ruckert B, et al. Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. J Allergy Clin Immunol. 2004 Dec; 114(6):1425-33.

120. Stelmach I, Jerzynska J, Bobrowska M, Brzozowska A, Majak P, Kuna P. [IL-10 serum levels in children with moderate asthma]. Pneumonol Alergol Pol. 2002; 70(1-2):25-33.

121. Hawrylowicz C, Richards D, Loke TK, Corrigan C, Lee T. A defect in corticosteroid-induced IL-10 production in T lymphocytes from corticosteroid-resistant asthmatic patients. J Allergy Clin Immunol. 2002 Feb; 109(2):369-70.

122. Thunberg S, Akdis M, Akdis CA, Grönneberg R, Malmström V, Trollmo C, et al. Immune regulation by CD4+CD25+ T cells and interleukin-10 in birch pollen-allergic patients and non-allergic controls. Clin Exp Allergy. 2007 Aug; 37(8):1127-36.

123. Jutel M, Akdis CA. T-cell regulatory mechanisms in specific immunotherapy. Chemical Immunology and Allergy. 2008; 94:158-77.

124. Meiler F, Klunker S, Zimmermann M, Akdis CA, Akdis M. Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors. Allergy. 2008 Nov; 63(11):1455-63.

125. Onishi Y, Fehervari Z, Yamaguchi T, Sakaguchi S. Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation. Proceedings of the National Academy of Sciences of the United States of America. 2008 Jul 22; 105(29):10113-8.

126. Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. The Journal of Experimental Medicine. 2000 Jul 17; 192(2):295-302.

127. Joetham A, Takeda K, Miyahara N, Matsubara S, Ohnishi H, Koya T, et al. Activation of naturally occurring lung CD4(+)CD25(+) regulatory T cells requires CD8 and MHC I interaction. Proceedings of the National Academy of Sciences of the United States of America. 2007 Sep 18; 104(38):15057-62.

128. Kearley J, Barker JE, Robinson DS, Lloyd CM. Resolution of airway inflammation and hyperreactivity after in vivo transfer of CD4+CD25+ regulatory T cells is interleukin 10 dependent. The Journal of Experimental Medicine. 2005 Dec 5; 202(11):1539-47.

129. Leech MD, Benson RA, De Vries A, Fitch PM, Howie SE. Resolution of Der p1-induced allergic airway inflammation is dependent on CD4+CD25+Foxp3+ regulatory cells. Journal of Immunology. 2007 Nov 15; 179(10):7050-8.

130. Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X, et al. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity. 2008 Apr; 28(4):546-58.

131. Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, et al. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. J Immunol. 2004 Mar; 172(5):3252-9.

132. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. The Journal of Clinical Investigation. 1998 Jul 1; 102(1):98-106.

133. Liu W, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, Zhu S, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of

human CD4+ T reg cells. The Journal of Experimental Medicine. 2006 Jul 10; 203(7):1701-11.

134. Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. CD4+CD25high regulatory cells in human peripheral blood. Journal of Immunology. 2001 Aug 1; 167(3):1245-53.

135. Tang Q, Boden EK, Henriksen KJ, Bour-Jordan H, Bi M, Bluestone JA. Distinct roles of CTLA-4 and TGF-beta in CD4+CD25+ regulatory T cell function. European Journal of Immunology. 2004 Nov; 34(11):2996-3005.

136. Ronchetti S, Zollo O, Bruscoli S, Agostini M, Bianchini R, Nocentini G, et al. GITR, a member of the TNF receptor superfamily, is costimulatory to mouse T lymphocyte subpopulations. European Journal of Immunology. 2004 Mar; 34(3):613-22.

137. Kolls JK, Lindén A. Interleukin-17 family members and inflammation. Immunity. 2004 Oct; 21(4):467-76.

138. Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. Th17: An Effector CD4 T Cell Lineage with Regulatory T Cell Ties. Immunity. 2006; 24(6):677-88.

139. Oda N, Canelos PB, Essayan DM, Plunkett BA, Myers AC, Huang SK. Interleukin-17F induces pulmonary neutrophilia and amplifies antigen-induced allergic response. Am J Respir Crit Care Med. 2005 Jan; 171(1):12-8.

140. Schnyder B, Schnyder-Candrian S, Pansky A, Schmitz ML, Heim M, Ryffel B, et al. IL-17 reduces TNF-induced Rantes and VCAM-1 expression. Cytokine. 2005 Aug 7; 31(3):191-202.

141. Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, et al. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. Immunity. 2002 Sep; 17(3):375-87.

142. Corren J. Cytokine inhibition in severe asthma: current knowledge and future directions. Curr Opin Pulm Med. 2011 Jan; 17(1):29-33.

143. Zhang N, Van Zele T, Perez-Novo C, Van Bruaene N, Holtappels G, DeRuyck N, et al. Different types of T-effector cells orchestrate mucosal inflammation in chronic sinus disease. J Allergy Clin Immunol. 2008 Nov; 122(5):961-8.

144. Laurence A, Tato CM, Davidson TS, Kanno Y, Chen Z, Yao Z, et al. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. Immunity. 2007 Mar; 26(3):371-81.

145. Zhou L, Lopes JE, Chong MM, Ivanov, II, Min R, Victora GD, et al. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature. 2008 May 8; 453(7192):236-40.

146. Du J, Huang C, Zhou B, Ziegler SF. Isoform-specific inhibition of ROR alpha-mediated transcriptional activation by human FOXP3. Journal of Immunology. 2008 Apr 1; 180(7):4785-92.

147. Godfrey DI, Berzins SP. Control points in NKT-cell development. Nat Rev Immunol. 2007 Jul; 7(7):505-18.

148. Stetson DB, Mohrs M, Reinhardt RL, Baron JL, Wang ZE, Gapin L, et al. Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells

poised for rapid effector function. J Exp Med. 2003 Oct; 198(7):1069-76.

149. Akbari O. The role of iNKT cells in development of bronchial asthma: a translational approach from animal models to human. Allergy. 2006 Aug; 61(8):962-8.

150. Vijayanand P, Seumois G, Pickard C, Powell RM, Angco G, Sammut D, et al. Invariant natural killer T cells in asthma and chronic obstructive pulmonary disease. N Engl J Med. 2007 Apr; 356(14):1410-22.

151. Mutalithas K, Croudace J, Guillen C, Siddiqui S, Thickett D, Wardlaw A, et al. Bronchoalveolar lavage invariant natural killer T cells are not increased in asthma. J Allergy Clin Immunol. 2007 May; 119(5):1274-6.

152. Holt PG, Schon-Hegrad MA, Oliver J, Holt BJ, McMenamin PG. A contiguous network of dendritic antigen-presenting cells within the respiratory epithelium. Int Arch Allergy Appl Immunol. 1990; 91(2):155-9.

153. Takano K, Kojima T, Go M, Murata M, Ichimiya S, Himi T, et al. HLA-DR- and CD11c-positive dendritic cells penetrate beyond well-developed epithelial tight junctions in human nasal mucosa of allergic rhinitis. J Histochem Cytochem. 2005 May; 53(5):611-9.

154. KleinJan APa, Willart MBa, van Rijt LSPa, Braunstahl G-JMDPa, Leman KBa, Jung SPb, et al. An essential role for dendritic cells in human and experimental allergic rhinitis. Journal of Allergy & Clinical Immunology. 2006; 118(5):1117-25.

155. Ito T, Inaba M, Inaba K, Toki J, Sogo S, Iguchi T, et al. A CD1a+/CD11c+ Subset of Human Blood Dendritic Cells Is a Direct

Precursor of Langerhans Cells. J Immunol. 1999 August 1, 1999; 163(3):1409-19.

156. Zuniga EI, McGavern DB, Pruneda-Paz JL, Teng C, Oldstone MB. Bone marrow plasmacytoid dendritic cells can differentiate into myeloid dendritic cells upon virus infection. Nat Immunol. 2004 Dec; 5(12):1227-34.

157. Dalod M, Hamilton T, Salomon R, Salazar-Mather TP, Henry SC, Hamilton JD, et al. Dendritic Cell Responses to Early Murine Cytomegalovirus Infection: Subset Functional Specialization and Differential Regulation by Interferon {alpha}/{beta}. J Exp Med. 2003 April 7, 2003; 197(7):885-98.

158. Van Rijt LS, Lambrecht BN. Dendritic cells in asthma: a function beyond sensitization. Clinical & Experimental Allergy. 2005; 35(9):1125-34.

159. Vermaelen KY, Carro-Muino I, Lambrecht BN, Pauwels RA. Specific Migratory Dendritic Cells Rapidly Transport Antigen from the Airways to the Thoracic Lymph Nodes. J Exp Med. 2000 December 27, 2000; 193(1):51-60.

160. Lambrecht BN, De Veerman M, Coyle AJ, Gutierrez-Ramos JC, Thielemans K, Pauwels RA. Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. J Clin Invest. 2000 Aug; 106(4):551-9.

161. Maldonado RA, Irvine DJ, Schreiber R, Glimcher LH. A role for the immunological synapse in lineage commitment of CD4 lymphocytes. Nature. 2004; 431(7008):527.

162. Kapsenberg ML. Dendritic-cell control of pathogen-driven T-cell polarization. Nat Rev Immunol. 2003 Dec; 3(12):984-93.

163. Balkwill FR, Burke F. The cytokine network. Immunol Today. 1989 Sep; 10(9):299-304.

164. Arai K, Tsuruta L, Watanabe S, Arai N. Cytokine signal networks and a new era in biomedical research. Mol Cells. 1997 Feb 28; 7(1):1-12.

165. Pawankar R, Okuda M, Yssel H, Okumura K, Ra C. Nasal mast cells in perennial allergic rhinitics exhibit increased expression of the Fc epsilonRI, CD40L, IL-4, and IL-13, and can induce IgE synthesis in B cells. J Clin Invest. 1997 Apr 1; 99(7):1492-9.

166. Ying S, Durham SR, Barkans J, Masuyama K, Jacobson M, Rak S, et al. T cells are the principal source of interleukin-5 mRNA in allergen-induced rhinitis. Am J Respir Cell Mol Biol. 1993 Oct; 9(4):356-60.

167. Bradding P, Feather IH, Wilson S, Bardin PG, Heusser CH, Holgate ST, et al. 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. 1993 Oct 1; 151(7):3853-65.

168. Kay AB, Ying S, Durham SR. Phenotype of cells positive for interleukin-4 and interleukin-5 mRNA in allergic tissue reactions. Int Arch Allergy Immunol. 1995 May-Jun; 107(1-3):208-10.

169. Bousquet J, van Cauwenberge P, Ait Khaled N, Bachert C, Baena-Cagnani CE, Bouchard J, et al. Pharmacologic and anti-IgE treatment of allergic rhinitis ARIA update (in collaboration with GA2LEN). Allergy. 2006; 61(9):1086-96.

170. Dubucquoi S, Desreumaux P, Janin A, Klein O, Goldman M, Tavernier J, et al. Interleukin 5 synthesis by eosinophils: association with granules and immunoglobulin-dependent secretion. J Exp Med. 1994 Feb 1; 179(2):703-8.

171. Giembycz MA, Lindsay MA. Pharmacology of the eosinophil. Pharmacol Rev. 1999 Jun; 51(2):213-340.

172. Salvi S, Semper A, Blomberg A, Holloway J, Jaffar Z, Papi A, et al. Interleukin-5 production by human airway epithelial cells. Am J Respir Cell Mol Biol. 1999 May; 20(5):984-91.

173. Clutterbuck EJ, Hirst EM, Sanderson CJ. 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 GMCSF. Blood. 1989 May 1; 73(6):1504-12.

174. Yamaguchi Y, Suda T, Suda J, Eguchi M, Miura Y, Harada N, et al. Purified interleukin 5 supports the terminal differentiation and proliferation of murine eosinophilic precursors. J Exp Med. 1988 Jan 1; 167(1):43-56.

175. Lopez AF, Shannon MF, Chia MM, Park L, Vadas MA. Regulation of human eosinophil production and function by interleukin-5. Immunol Ser. 1992; 57:549-71.

176. Clutterbuck EJ, Sanderson CJ. Regulation of human eosinophil precursor production by cytokines: a comparison of recombinant human interleukin-1 (rhIL-1), rhIL-3, rhIL-5, rhIL-6, and rh granulocyte-macrophage colony-stimulating factor. Blood. 1990 May 1; 75(9):1774-9.

177. Resnick MB, Weller PF. Mechanisms of eosinophil recruitment. American Journal of Respiratory Cell and Molecular Biology. 1993 Apr; 8(4):349-55.

178. Warringa RA, Schweizer RC, Maikoe T, Kuijper PH, Bruijnzeel PL, Koendermann L. Modulation of eosinophil chemotaxis by interleukin-5. American Journal of Respiratory Cell and Molecular Biology. 1992 Dec; 7(6):631-6.

179. Carlson M, Peterson C, Venge P. The influence of IL-3, IL-5, and GM-CSF on normal human eosinophil and neutrophil C3b-induced degranulation. Allergy. 1993 Aug; 48(6):437-42.

180. Kita H, Weiler DA, Abu-Ghazaleh R, Sanderson CJ, Gleich GJ. Release of granule proteins from eosinophils cultured with IL-5. J Immunol. 1992 Jul 15; 149(2):629-35.

181. Yamaguchi Y, Suda T, Ohta S, Tominaga K, Miura Y, Kasahara T. Analysis of the survival of mature human eosinophils: interleukin-5 prevents apoptosis in mature human eosinophils. Blood. 1991 Nov 15; 78(10):2542-7.

182. Egan RW, Athwal D, Bodmer MW, Carter JM, Chapman RW, Chou CC, et al. Effect of Sch 55700, a humanized monoclonal antibody to human interleukin-5, on eosinophilic responses and bronchial hyperreactivity. Arzneimittel-Forschung. 1999 Sep; 49(9):779-90.

183. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. Lancet. 2000 Dec 23-30; 356(9248):2144-8.

184. Greenfeder S, Umland SP, Cuss FM, Chapman RW, Egan RW. Th2 cytokines and asthma. The role of interleukin-5 in allergic eosinophilic disease. Respir Res. 2001; 2(2):71-9.

185. Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. N Engl J Med. 2009 Mar 5; 360(10):985-93.

186. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. N Engl J Med. 2009 Mar 5; 360(10):973-84.

187. Trautmann A, Altznauer F, Akdis M, Simon HU, Disch R, Brocker EB, et al. The differential fate of cadherins during T-cell-induced keratinocyte apoptosis leads to spongiosis in eczematous dermatitis. J Invest Dermatol. 2001 Oct; 117(4):927-34.

188. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001; 19:683-765.

189. Williams LM, Ricchetti G, Sarma U, Smallie T, Foxwell BMJ. Interleukin-10 suppression of myeloid cell activation - a continuing puzzle. Immunology. 2004; 113(3):281-92.

190. Pretolani M. Interleukin-10: an anti-inflammatory cytokine with therapeutic potential. Clin Exp Allergy. 1999 Sep; 29(9):1164-71.

191. Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy--review of a new approach. Pharmacol Rev. 2003 Jun; 55(2):241-69.

192. de Waal Malefyt R, Yssel H, de Vries JE. Direct effects of IL-10 on subsets of human CD4+ T cell clones and resting T cells. Specific

inhibition of IL-2 production and proliferation. J Immunol. 1993 Jun 1; 150(11):4754-65.

193. Taga K, Mostowski H, Tosato G. Human interleukin-10 can directly inhibit T-cell growth. Blood. 1993 Jun 1; 81(11):2964-71.

194. Schandene L, Alonso-Vega C, Willems F, Gerard C, Delvaux A, Velu T, et al. B7/CD28-dependent IL-5 production by human resting T cells is inhibited by IL-10. J Immunol. 1994 May 1; 152(9):4368-74.

195. Akdis CA, Blaser K. Mechanisms of interleukin-10-mediated immune suppression. Immunology. 2001; 103(2):131-6.

196. Finbloom DS, Winestock KD. IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential assembly of STAT1 alpha and STAT3 complexes in human T cells and monocytes. J Immunol. 1995 Aug 1; 155(3):1079-90.

197. Punnonen J, de Waal Malefyt R, van Vlasselaer P, Gauchat JF, de Vries JE. IL-10 and viral IL-10 prevent IL-4-induced IgE synthesis by inhibiting the accessory cell function of monocytes. J Immunol. 1993 Aug 1; 151(3):1280-9.

198. Ding L, Linsley PS, Huang LY, Germain RN, Shevach EM. IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. J Immunol. 1993 Aug 1; 151(3):1224-34.

199. Takanaski S, Nonaka R, Xing Z, O'Byrne P, Dolovich J, Jordana M. Interleukin 10 inhibits lipopolysaccharide-induced survival and cytokine production by human peripheral blood eosinophils. J Exp Med. 1994 Aug 1; 180(2):711-5.

200. Ohkawara Y, Lim KG, Xing Z, Glibetic M, Nakano K, Dolovich J, et al. CD40 expression by human peripheral blood eosinophils. J Clin Invest. 1996 Apr 1; 97(7):1761-6.

201. Joyce DA, Ilett KF. Therapeutics. Med J Aust. 1994 Nov 21; 161(10):622-6.

202. Jenkins JK, Malyak M, Arend WP. The effects of interleukin-10 on interleukin-1 receptor antagonist and interleukin-1 beta production in human monocytes and neutrophils. Lymphokine Cytokine Res. 1994 Feb; 13(1):47-54.

203. Lacraz S, Nicod LP, Chicheportiche R, Welgus HG, Dayer JM. IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. J Clin Invest. 1995 Nov; 96(5):2304-10.

204. Akdis M, Burgler S, Crameri R, Eiwegger T, Fujita H, Gomez E, et al. Interleukins, from 1 to 37, and interferon-gamma: receptors, functions, and roles in diseases. J Allergy Clin Immunol. 2011 Mar; 127(3):701-21 e1-70.

205. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. Journal of Leukocyte Biology. 2004 Feb; 75(2):163-89.

206. Corthay A. A three-cell model for activation of naive T helper cells. Scandinavian Journal of Immunology. 2006 Aug; 64(2):93-6.

207. Morgan DA, Ruscetti FW, Gallo R. Selective in vitro growth of T lymphocytes from normal human bone marrows. Science. 1976 Sep 10; 193(4257):1007-8.

208. Malek TR. The biology of interleukin-2. Annual Review of Immunology. 2008; 26:453-79.

209. Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell. 2001 Feb 23; 104(4):487-501.

210. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. 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. 1992 Apr 15; 148(8):2390-4.

211. Sim TC, Grant JA, Hilsmeier KA, Fukuda Y, Alam R. Proinflammatory cytokines in nasal secretions of allergic subjects after antigen challenge. Am J Respir Crit Care Med. 1994 Feb; 149(2 Pt 1):339-44.

212. Terada N, Konno A, Fukuda S, Yamashita T, Shirotori K, Okamoto Y, et al. Interleukin-5 gene expression in nasal mucosa and changes in amount of interleukin-5 in nasal lavage fluid after antigen challenge. Acta Otolaryngol. 1994 Mar; 114(2):203-8.

213. Minshall EM, Cameron L, Lavigne F, Leung DY, Hamilos D, Garcia-Zepada EA, et al. Eotaxin mRNA and protein expression in chronic sinusitis and allergen-induced nasal responses in seasonal allergic rhinitis. Am J Respir Cell Mol Biol. 1997 Dec; 17(6):683-90.

214. Alam R, Sim TC, Hilsmeier K, Grant JA. Development of a new technique for recovery of cytokines from inflammatory sites in situ. J Immunol Methods. 1992 Oct 19; 155(1):25-9.

215. Ghaffar O, Laberge S, Jacobson MR, Lowhagen O, Rak S, Durham SR, et al. IL-13 mRNA and immunoreactivity in allergen-induced rhinitis: comparison with IL-4 expression and modulation by topical glucocorticoid therapy. Am J Respir Cell Mol Biol. 1997 Jul; 17(1):17-24.

216. Ying S, Durham SR, Jacobson MR, Rak S, Masuyama K, Lowhagen O, et al. T lymphocytes and mast cells express messenger RNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. Immunology. 1994 Jun; 82(2):200-6.

217. Saito H, Asakura K, Ogasawara H, Watanabe M, Kataura A. Topical antigen provocation increases the number of immunoreactive IL-4-, IL-5- and IL-6-positive cells in the nasal mucosa of patients with perennial allergic rhinitis. Int Arch Allergy Immunol. 1997 Sep; 114(1):81-5.

218. Bartemes KR, Cooper KM, Drain KL, Kita H. Secretory IgA induces antigen-independent eosinophil survival and cytokine production without inducing effector functions. J Allergy Clin Immunol. 2005 Oct; 116(4):827-35.

219. Fan GK, Wang H, Takenaka H. Eosinophil infiltration and activation in nasal polyposis. Acta Otolaryngol. 2007 May; 127(5):521-6.

220. Akdis M, Verhagen J, Taylor A, Karamloo F, Karagiannidis C, Crameri R, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. J Exp Med. 2004 Jun; 199(11):1567-75.

221. Boyce JA, Austen KF. No audible wheezing: nuggets and conundrums from mouse asthma models. The Journal of Experimental Medicine. 2005 Jun 20; 201(12):1869-73.

222. Akbari O, Freeman G, J, Meyer E, H, Greenfield E, A, Chang T, T, Sharpe A, H, et al. Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. Nature Medicine. 2002; 8(9):1024.

223. Strickland DH, Stumbles PA, Zosky GR, Subrata LS, Thomas JA, Turner DJ, et al. Reversal of airway hyperresponsiveness by induction of airway mucosal CD4+CD25+ regulatory T cells. The Journal of Experimental Medicine. 2006 Nov 27; 203(12):2649-60.

224. Lewkowich IP, Herman NS, Schleifer KW, Dance MP, Chen BL, Dienger KM, et al. CD4+CD25+ T cells protect against experimentally induced asthma and alter pulmonary dendritic cell phenotype and function. The Journal of Experimental Medicine. 2005 Dec 5; 202(11):1549-61.

225. Ostroukhova M, Seguin-Devaux C, Oriss TB, Dixon-McCarthy B, Yang L, Ameredes BT, et al. Tolerance induced by inhaled antigen involves CD4(+) T cells expressing membrane-bound TGF-beta and FOXP3. The Journal of Clinical Investigation. 2004 Jul; 114(1):28-38.

226. Thunberg S, Akdis M, Akdis CA, Gronneberg R, Malmstrom V, Trollmo C, et al. Immune regulation by CD4+CD25+ T cells and interleukin-10 in birch pollen-allergic patients and non-allergic controls. Clinical and Experimental Allergy : Journal of the British Society for Allergy and Clinical Immunology. 2007 Aug; 37(8):1127-36.

227. Taams LS, Vukmanovic-Stejic M, Smith J, Dunne PJ, Fletcher JM, Plunkett FJ, et al. Antigen-specific T cell suppression by human CD4+CD25+ regulatory T cells. European Journal of Immunology. 2002 Jun; 32(6):1621-30.

228. Borish L, Aarons A, Rumbyrt J, Cvietusa P, Negri J, Wenzel S. Interleukin-10 regulation in normal subjects and patients with asthma. J Allergy Clin Immunol. 1996 Jun; 97(6):1288-96.

229. Lim S, Crawley E, Woo P, Barnes PJ. Haplotype associated with low interleukin-10 production in patients with severe asthma. Lancet. 1998 Jul 11; 352(9122):113.

230. Karagiannidis C, Akdis M, Holopainen P, Woolley NJ, Hense G, Ruckert B, et al. Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. J Allergy Clin Immunol. 2004 Dec; 114(6):1425-33.

231. Xystrakis E, Kusumakar S, Boswell S, Peek E, Urry Z, Richards DF, et al. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. The Journal of Clinical Investigation. 2006 Jan; 116(1):146-55.

232. Prigione I, Morandi F, Tosca MA, Silvestri M, Pistoia V, Ciprandi G, et al. Interferon-gamma and IL-10 may protect from allergic polysensitization in children: preliminary evidence. Allergy. 2010 Jun; 65(6):740-2.

233. Borrego LM, Arroz MJ, Videira P, Martins C, Guimarães H, Nunes G, et al. Regulatory cells, cytokine pattern and clinical risk factors for asthma

in infants and young children with recurrent wheeze. Clin Exp Allergy. 2009 Aug; 39(8):1160-9.

234. Cameron L, Hamid Q, Wright E, Nakamura Y, Christodoulopoulos P, Muro S, et al. Local synthesis of epsilon germline gene transcripts, IL-4, and IL-13 in allergic nasal mucosa after ex vivo allergen exposure. J Allergy Clin Immunol. 2000 Jul; 106(1 Pt 1):46-52.

235. Zuany-Amorim C, Haile S, Leduc D, Dumarey C, Huerre M, Vargaftig BB, et al. Interleukin-10 inhibits antigen-induced cellular recruitment into the airways of sensitized mice. The Journal of Clinical Investigation. 1995 Jun; 95(6):2644-51.

236. John M, Lim S, Seybold J, Jose P, Robichaud A, O'Connor B, et al. Inhaled corticosteroids increase interleukin-10 but reduce macrophage inflammatory protein-1alpha, granulocyte-macrophage colony-stimulating factor, and interferon-gamma release from alveolar macrophages in asthma. Am J Respir Crit Care Med. 1998 Jan; 157(1):256-62.

237. Hartl D, Koller B, Mehlhorn AT, Reinhardt D, Nicolai T, Schendel DJ, et al. Quantitative and functional impairment of pulmonary CD4+CD25hi regulatory T cells in pediatric asthma. J Allergy Clin Immunol. 2007 May; 119(5):1258-66.

238. Xu G, Mou Z, Jiang H, Cheng L, Shi J, Xu R, et al. A possible role of CD4+CD25+ T cells as well as transcription factor Foxp3 in the dysregulation of allergic rhinitis. The Laryngoscope. 2007 May; 117(5):876-80.

239. Radulovic S, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4+ CD25+ cells in the nasal mucosa. J Allergy Clin Immunol. 2008 Jun; 121(6):1467-72, 72 e1.

240. Rinia AB, Kostamo K, Ebbens FA, van Drunen CM, Fokkens WJ. Nasal polyposis: a cellular-based approach to answering questions. Allergy. 2007; 62(4):348-58.

241. Fokkens W, Lund V, Mullol J. EP3OS 2007: European position paper on rhinosinusitis and nasal polyps 2007. A summary for otorhinolaryngologists. Rhinology. 2007 Jun; 45(2):97-101.

242. Bateman ND, Fahy C, Woolford TJ. Nasal polyps: still more questions than answers. J Laryngol Otol. 2003 Jan; 117(1):1-9.

243. Settipane G. Nasal Polyps: Epidemiology, Pathology, Immunology, and Treatment. American Journal of Rhinology. 1987; 1:119-26.

244. Greisner WA, 3rd, Settipane GA. Hereditary factor for nasal polyps. Allergy Asthma Proc. 1996 Sep-Oct; 17(5):283-6.

245. Settipane GA. Epidemiology of nasal polyps. Allergy Asthma Proc. 1996 Sep-Oct; 17(5):231-6.

246. Maran AGD, Lund VJ. Nasal Polyps. In: Maran AGD, Lund VJ, editors. Clinical rhinology. New York: Thieme; 1990. p. 94-8.

247. Frenkiel S, Small P. Pathogenesis and treatment of nasal polyps. In: Blitzer A, Lawson W, Friedman WH, editors. Surgery of the paranasal sinuses. Philadelphia: WB Saunders; 1991. p. 41-9.

248. Drake-Lee AB. Nasal Polyps. In: Kerr AG, Groves J, editors. Scott-Brown's Otolaryngology. London: Butterworths; 1987. p. 142-53.

249. Schapowal AG, Simon HU, Schmitz-Schumann M. Phenomenology, pathogenesis, diagnosis and treatment of aspirin-sensitive rhinosinusitis. Acta Otorhinolaryngol Belg. 1995; 49(3):235-50.

250. Sonneville A. [Asthma and aspirin]. Allerg Immunol (Paris). 1998 Apr; 30(4):117-9.

251. Moneret-Vautrin DA, Jankowski R, Bene MC, Kanny G, Hsieh V, Faure G, et al. NARES: a model of inflammation caused by activated eosinophils? Rhinology. 1992 Sep; 30(3):161-8.

252. Schiavino D, Nucera E, Milani A, Della Corte AM, D'Ambrosio C, Pagliari G, et al. Nasal lavage cytometry in the diagnosis of nonallergic rhinitis with eosinophilia syndrome (NARES). Allergy Asthma Proc. 1997 Nov-Dec; 18(6):363-6.

253. Afzelius BA. Disorders of ciliary motility. Hosp Pract (Off Ed). 1986 Mar 15; 21(3):73-80.

254. Rossman CM, Lee RM, Forrest JB, Newhouse MT. Nasal ciliary ultrastructure and function in patients with primary ciliary dyskinesia compared with that in normal subjects and in subjects with various respiratory diseases. Am Rev Respir Dis. 1984 Jan; 129(1):161-7.

255. Jamal A, Maran AGD. Atopy and nasal polyposis. The Journal of Laryngology & Otology. 1987; 101(04):355-8.

256. Settipane GA, Chafee FH. Nasal polyps in asthma and rhinitis. A review of 6,037 patients. J Allergy Clin Immunol. 1977 Jan; 59(1):17-21.

257. Wong D, Dolovich J. Blood Eosinophilia and Nasal Polyps. American Journal of Rhinology. 1992; 6:195-8.

258. Bachert C, Gevaert P, Holtappels G, Johansson SG, van Cauwenberge P. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. J Allergy Clin Immunol. 2001 Apr; 107(4):607-14.

259. Mygind N, Dahl R, Bachert C. Nasal polyposis, eosinophil dominated inflammation, and allergy. Thorax. 2000 Oct; 55 Suppl 2:S79-83.

260. Grevers G, Stammberger H. Sinusitis und Polyposis nasi. In: Grevers G, editor. Praktische Rhinologie. München: Urban und Schwarzenberg; 1998. p. 137-52.

261. Kim JW, Hong SL, Kim YK, Lee CH, Min YG, Rhee CS. Histological and immunological features of non-eosinophilic nasal polyps. Otolaryngology--Head and Neck Surgery. 2007 Dec; 137(6):925-30.

262. Shen Y, Tang XY, Yang YC, Ke X, Kou W, Pan CK, et al. Impaired balance of Th17/Treg in patients with nasal polyposis. Scandinavian Journal of Immunology. 2011 Aug; 74(2):176-85.

263. Van Zele T, Claeys S, Gevaert P, Van Maele G, Holtappels G, Van Cauwenberge P, et al. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. Allergy. 2006 Nov; 61(11):1280-9.

264. Bachert C, Gevaert P, van Cauwenberge P. Nasal Polyposis - A New Concept on the Formation of Polyps. Allergy Clin Immunol Int: J World Allergy Org. 1999 Jul; 11(4):130-5.

265. Pawliczak R, Lewandowska-Polak A, Kowalski ML. Pathogenesis of nasal polyps: an update. Curr Allergy Asthma Rep. 2005 Nov; 5(6):463-71.

266. Bernstein JM. The molecular biology of nasal polyposis. Curr Allergy Asthma Rep. 2001 May; 1(3):262-7.

267. Gevaert P, Bachert C, Holtappels G, Novo CP, Van der Heyden J, Fransen L, et al. Enhanced soluble interleukin-5 receptor alpha expression in nasal polyposis. Allergy. 2003; 58(5):371-9.

268. Ruhno J, Howie K, Anderson M, Andersson B, Vanzieleghem M, Hitch D, et al. The increased number of epithelial mast cells in nasal polyps and adjacent turbinates is not allergy-dependent. Allergy. 1990 Jul; 45(5):370-4.

269. Kawabori S, Denburg JA, Schwartz LB, Irani AA, Wong D, Jordana G, et al. Histochemical and immunohistochemical characteristics of mast cells in nasal polyps. Am J Respir Cell Mol Biol. 1992 Jan; 6(1):37-43.

270. Sasaki Y. Distribution of the degranulated and non-degranulated mast cells in nasal polyp. Acta Otolaryngol Suppl. 1986; 430:34-8.

271. Coste A, Wang QP, Roudot-Thoraval F, Chapelin C, Bedbeder P, Poron F, et al. Epithelial cell proliferation in nasal polyps could be up-regulated by platelet-derived growth factor. Laryngoscope. 1996 May; 106(5 Pt 1):578-83.

272. Ohno I, Lea RG, Flanders KC, Clark DA, Banwatt D, Dolovich J, et al. Eosinophils in chronically inflamed human upper airway tissues express transforming growth factor beta 1 gene (TGF beta 1). J Clin Invest. 1992 May; 89(5):1662-8.

273. Eisma RJ, Allen JS, Lafreniere D, Leonard G, Kreutzer DL. Eosinophil expression of transforming growth factor-beta and its receptors in nasal

polyposis: role of the cytokines in this disease process. Am J Otolaryngol. 1997 Nov-Dec; 18(6):405-11.

274. Nakagawa T, Yamane H, Shigeta T, Takashima T, Nakai Y. Interaction between fibronectin and eosinophils in the growth of nasal polyps. Laryngoscope. 1999 Apr; 109(4):557-61.

275. Bachert C, Gevaert P, Holtappels G, Cuvelier C, van Cauwenberge P. Nasal polyposis: from cytokines to growth. Am J Rhinol. 2000 Sep-Oct; 14(5):279-90.

276. Gevaert P, Holtappels G, Johansson SG, Cuvelier C, Cauwenberge P, Bachert C. Organization of secondary lymphoid tissue and local IgE formation to Staphylococcus aureus enterotoxins in nasal polyp tissue. Allergy. 2005 Jan; 60(1):71-9.

277. Shoham T, Yaniv E, Koren R, Gal R, Parameswaran R, Kravitz A, et al. Reduced expression of activin A in focal lymphoid agglomerates within nasal polyps. J Histochem Cytochem. 2001 Oct; 49(10):1245-52.

278. Gould HJ, Takhar P, Harries HE, Durham SR, Corrigan CJ. Germinal-centre reactions in allergic inflammation. Trends Immunol. 2006 Oct; 27(10):446-52.

279. Chapelin C, Coste A, Gilain L, Poron F, Verra F, Escudier E. Modified epithelial cell distribution in chronic airways inflammation. Eur Respir J. 1996 Dec; 9(12):2474-8.

280. Kakoi H, Hiraide F. A histological study of formation and growth of nasal polyps. Acta Oto-Laryngologica. 1987 Jan-Feb; 103(1-2):137-44.

281. Kramer MF, Rasp G. Nasal polyposis: eosinophils and interleukin-5. Allergy. 1999; 54(7):669-80.

282. Jankowski R. Eosinophils in the pathophysiology of nasal polyposis. Acta Otolaryngol. 1996 Mar; 116(2):160-3.

283. Pawankar R. Nasal polyposis: an update: editorial review. Curr Opin Allergy Clin Immunol. 2003 Feb; 3(1):1-6.

284. Allen JS, Eisma R, Leonard G, Lafreniere D, Kreutzer D. Interleukin-8 expression in human nasal polyps. Otolaryngol Head Neck Surg. 1997 Nov; 117(5):535-41.

285. Figueiredo CRa, Silva IDCGb, Weckx LLMa. Inflammatory genes in nasal polyposis. Current Opinion in Otolaryngology & Head & Neck Surgery. 2008; 16(1):18-21.

286. Min YG, Jung HW, Kim HS, Park SK, Yoo KY. Prevalence and risk factors of chronic sinusitis in Korea: results of a nationwide survey. Eur Arch Otorhinolaryngol. 1996; 253(7):435-9.

287. Lee CH, Rhee CS, Min YG. Cytokine gene expression in nasal polyps. Ann Otol Rhinol Laryngol. 1998 Aug; 107(8):665-70.

288. Kramer MF, Ostertag P, Pfrogner E, Rasp G. Nasal interleukin-5, immunoglobulin E, eosinophilic cationic protein, and soluble intercellular adhesion molecule-1 in chronic sinusitis, allergic rhinitis, and nasal polyposis. Laryngoscope. 2000 Jun; 110(6):1056-62.

289. Kramer MFMD, Ostertag PMD, Pfrogner E, Rasp GMD. Nasal Interleukin-5, Immunoglobulin E, Eosinophilic Cationic Protein, and Soluble Intercellular Adhesion Molecule-1 in Chronic Sinusitis, Allergic Rhinitis, and Nasal Polyposis. Laryngoscope. 2000; 110(6):1056-62.

290. Eliashar R, Levi-Schaffer F. The role of the eosinophil in nasal diseases. Curr Opin Otolaryngol Head Neck Surg. 2005 Jun; 13(3):171-5.

291. Bachert C, Van Cauwenberge PB. Inflammatory mechanisms in chronic sinusitis. Acta Otorhinolaryngol Belg. 1997; 51(4):209-17.

292. Allen JS, Eisma R, Leonard G, Kreutzer D. Interleukin-3 interleukin-5, and granulocyte-macrophage colony-stimulating factor expression in nasal polyps. Am J Otolaryngol. 1997 Jul-Aug; 18(4):239-46.

293. Min YG, Lee CH, Rhee CS, Kim KH, Kim CS, Koh YY, et al. Inflammatory cytokine expression on nasal polyps developed in allergic and infectious rhinitis. Acta Otolaryngol. 1997 Mar; 117(2):302-6.

294. Coste A, Lefaucheur JP, Wang QP, Lesprit E, Poron F, Peynegre R, et al. Expression of the transforming growth factor beta isoforms in inflammatory cells of nasal polyps. Arch Otolaryngol Head Neck Surg. 1998 Dec; 124(12):1361-6.

295. Denburg JA. Haemopoietic mechanisms in nasal polyposis and asthma. Thorax. 2000 Oct; 55 Suppl 2:S24-5.

296. Hamilos DL, Leung DY, Huston DP, Kamil A, Wood R, Hamid Q. GM-CSF, IL-5 and RANTES immunoreactivity and mRNA expression in chronic hyperplastic sinusitis with nasal polyposis (NP). Clinical and Experimental Allergy : Journal of the British Society for Allergy and Clinical Immunology. 1998 Sep; 28(9):1145-52.

297. Walsh GM. Eosinophil apoptosis: mechanisms and clinical relevance in asthmatic and allergic inflammation. Br J Haematol. 2000 Oct; 111(1):61-7.

298. Bachert C, Wagenmann M, Hauser U, Rudack C. IL-5 synthesis is upregulated in human nasal polyp tissue. J Allergy Clin Immunol. 1997 Jun; 99(6 Pt 1):837-42.

299. Simon HU, Yousefi S, Schranz C, Schapowal A, Bachert C, Blaser K. Direct demonstration of delayed eosinophil apoptosis as a mechanism causing tissue eosinophilia. J Immunol. 1997 Apr 15; 158(8):3902-8.

300. Egan RW, Umland SP, Cuss FM, Chapman RW. Biology of Interleukin-5 and its Relevance to Allergic Disease. Allergy. 1996; 51(2):71-81.

301. Simon HU. [Dysregulated apoptosis in chronic eosinophilic diseases--new therapeutic strategies for allergies and bronchial asthma]. Pneumologie. 1996 Nov; 50(11):790-6.

302. Terada N, Konno A, Natori T, Tada H, Togawa K. Interleukin-5 preferentially recruits eosinophils from vessels in nasal mucosa. Acta Otolaryngol Suppl. 1993; 506:57-60.

303. Simon HU. Molecular mechanisms of defective eosinophil apoptosis in diseases associated with eosinophilia. Int Arch Allergy Immunol. 1997 May-Jul; 113(1-3):206-8.

304. Bachert C, Gevaert P, Holtappels G, van Cauwenberge P. Mediators in nasal polyposis. Curr Allergy Asthma Rep. 2002 Nov; 2(6):481-7.

305. Bikhazi NB. Contemporary management of nasal polyps. Otolaryngol Clin North Am. 2004 Apr; 37(2):327-37, vi.

306. Danzig M, Cuss F. Inhibition of interleukin-5 with a monoclonal antibody attenuates allergic inflammation. Allergy. 1997 Aug; 52(8):787-94.

307. Shin SH, Lee SH, Jeong HS, Kita H. The effect of nasal polyp epithelial cells on eosinophil activation. Laryngoscope. 2003 Aug; 113(8):1374-7.

308. Weller PF. The immunobiology of eosinophils. N Engl J Med. 1991 Apr 18; 324(16):1110-8.

309. Hamilos DL, Leung DY, Wood R, Cunningham L, Bean DK, Yasruel Z, et al. Evidence for distinct cytokine expression in allergic versus nonallergic chronic sinusitis. J Allergy Clin Immunol. 1995 Oct; 96(4):537-44.

310. Miller CH, Pudiak DR, Hatem F, Looney RJ. Accumulation of interferon gamma-producing TH1 helper T cells in nasal polyps. Otolaryngol Head Neck Surg. 1994 Jul; 111(1):51-8.

311. Dellacono FR, Eisma R, Lafreniere D, Leonard G, Kreutzer D. Interferon gamma expression in human nasal polyps. Laryngoscope. 1997 May; 107(5):626-30.

312. Woodworth BA, Joseph K, Kaplan AP, Schlosser RJ. Alterations in eotaxin, monocyte chemoattractant protein-4, interleukin-5, and interleukin-13 after systemic steroid treatment for nasal polyps. Otolaryngol Head Neck Surg. 2004 Nov; 131(5):585-9.

313. Hamilos DL, Plout M, Leung DY, Huston DP, Hamid QA. GM-CSF, IL-5 and RANTES messenger RNA expression and protein levels in chronic hyperplastic sinusitis with nasal polyposis. Journal of Clinical Immunology. 1997; 99:130.

314. Allen JS, Eisma R, LaFreniere D, Leonard G, Kreutzer D. Characterization of the eosinophil chemokine RANTES in nasal polyps. Ann Otol Rhinol Laryngol. 1998 May; 107(5 Pt 1):416-20.

315. Bradley DT, Kountakis SE. Role of interleukins and transforming growth factor-beta in chronic rhinosinusitis and nasal polyposis. Laryngoscope. 2005 Apr; 115(4):684-6.

316. Watelet JB, Claeys C, Perez-Novo C, Gevaert P, Van Cauwenberge P, Bachert C. Transforming growth factor beta1 in nasal remodeling: differences between chronic rhinosinusitis and nasal polyposis. Am J Rhinol. 2004 Sep-Oct; 18(5):267-72.

317. Watelet JB, Gevaert P, Bachert C, Holtappels G, van Cauwenberge P. Secretion of TGF-betal, TGF-beta2, EGF and PDGF into nasal fluid after sinus surgery. Eur Arch Otorhinolaryngol. 2002 May; 259(5):234-8.

318. Holgate ST. Epithelial damage and response. Clin Exp Allergy. 2000 Jun; 30 Suppl 1:37-41.

319. Coste A, Brugel L, Maitre B, Boussat S, Papon JF, Wingerstmann L, et al. Inflammatory cells as well as epithelial cells in nasal polyps express vascular endothelial growth factor. Eur Respir J. 2000 Feb; 15(2):367-72.

320. Beck LA, Stellato C, Beall LD, Schall TJ, Leopold D, Bickel CA, et al. Detection of the chemokine RANTES and endothelial adhesion molecules in nasal polyps. J Allergy Clin Immunol. 1996 Oct; 98(4):766-80.

321. Hamilos DL, Leung DY, Wood R, Bean DK, Song YL, Schotman E, et al. Eosinophil infiltration in nonallergic chronic hyperplastic sinusitis with nasal polyposis (CHS/NP) is associated with endothelial VCAM-1

upregulation and expression of TNF-alpha. Am J Respir Cell Mol Biol. 1996 Oct; 15(4):443-50.

322. Symon FA, Walsh GM, Watson SR, Wardlaw AJ. Eosinophil adhesion to nasal polyp endothelium is P-selectin-dependent. J Exp Med. 1994 Jul 1; 180(1):371-6.

323. Hirschberg AMDP, Jokuti A, Darvas ZMDP, Almay KMD, Repassy GMDP, Falus APM. The Pathogenesis of Nasal Polyposis by Immunoglobulin E and Interleukin-5 Is Completed by Transforming Growth Factor-[beta]1. Laryngoscope. 2003; 113(1):120-4.

324. Drake-Lee A, Jones V, Lewin I, Nayyar S, Wells A, Stanworth D. Levels of substance P and IgE decapeptide in nasal polyp fluid and matching sera: a preliminary study. J Laryngol Otol. 1996 Mar; 110(3):225-7.

325. Abdelilah SG, Bouchaib L, Morita M, Delphine A, Marika S, Andre C, et al. Molecular characterization of the low-affinity IgE receptor Fc epsilonRII/CD23 expressed by human eosinophils. Int Immunol. 1998 Apr; 10(4):395-404.

326. Gounni AS, Lamkhioued B, Ochiai K, Tanaka Y, Delaporte E, Capron A, et al. High-affinity IgE receptor on eosinophils is involved in defence against parasites. Nature. 1994 Jan 13; 367(6459):183-6.

327. Van Zele T, Gevaert P, Watelet JB, Claeys G, Holtappels G, Claeys C, et al. Staphylococcus aureus colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. J Allergy Clin Immunol. 2004 Oct; 114(4):981-3.

328. Norlander T, Westrin KM, Fukami M, Stierna P, Carlsoo B. Experimentally induced polyps in the sinus mucosa: a structural analysis of the initial stages. Laryngoscope. 1996 Feb; 106(2 Pt 1):196-203.

329. Larsen PL, Tos M, Kuijpers W, van der Beek JM. The early stages of polyp formation. Laryngoscope. 1992 Jun; 102(6):670-7.

330. Caye-Thomasen P, Hermansson A, Tos M, Prellner K. Polyp pathogenesis--a histopathological study in experimental otitis media. Acta Otolaryngol. 1995 Jan; 115(1):76-82.

331. Larsen PL, Tos M. Origin of nasal polyps. Laryngoscope. 1991 Mar; 101(3):305-12.

332. Tos M, Sasaki Y, Ohnishi M, Larsen P, Drake-Lee AB. Fireside conference 2. Pathogenesis of nasal polyps. Rhinol Suppl. 1992; 14:181-5.

333. Mullol J, Fernandez-Morata JC, Roca-Ferrer J, Pujols L, Xaubet A, Benitez P, et al. Cyclooxygenase 1 and cyclooxygenase 2 expression is abnormally regulated in human nasal polyps. J Allergy Clin Immunol. 2002 May; 109(5):824-30.

334. Szczeklik A, Stevenson DD. Aspirin-induced asthma: advances in pathogenesis and management. J Allergy Clin Immunol. 1999 Jul; 104(1):5-13.

335. Grzanka A, Misiolek M, Golusinski W, Jarzab J. Molecular mechanisms of glucocorticoids action: implications for treatment of rhinosinusitis and nasal polyposis. Eur Arch Otorhinolaryngol. 2010 Jul 14.

336. Van Zele T, Gevaert P, Holtappels G, Beule A, Wormald PJ, Mayr S, et al. Oral steroids and doxycycline: two different approaches to treat nasal polyps. J Allergy Clin Immunol. 2010 May; 125(5):1069-76 e4.

337. Rasp G, Bujia J. [Treatment of nasal polyposis with systemic and local corticoids]. Acta Otorrinolaringol Esp. 1997 Jan-Feb; 48(1):37-40.

338. Drake-Lee AB. Medical treatment of nasal polyps. Rhinology. 1994 Mar; 32(1):1-4.

339. Lund VJ, Flood J, Sykes AP, Richards DH. Effect of fluticasone in severe polyposis. Arch Otolaryngol Head Neck Surg. 1998 May; 124(5):513-8.

340. Gevaert P, Lang-Loidolt D, Lackner A, Stammberger H, Staudinger H, Van Zele T, et al. Nasal IL-5 levels determine the response to anti-IL-5 treatment in patients with nasal polyps. J Allergy Clin Immunol. 2006 Nov; 118(5):1133-41.

341. Schäper C, Noga O, Koch B, Ewert R, Felix SB, Gläser S, et al. Anti-inflammatory properties of montelukast, a leukotriene receptor antagonist in patients with asthma and nasal polyposis. J Investig Allergol Clin Immunol. 2011; 21(1):51-8.

342. Fokkens W, Lund V, Bachert C, Clement P, Helllings P, Holmstrom M, et al. EAACI position paper on rhinosinusitis and nasal polyps executive summary. Allergy. 2005 May; 60(5):583-601.

343. Sanchez-Segura A, Brieva JA, Rodriguez C. T lymphocytes that infiltrate nasal polyps have a specialized phenotype and produce a mixed TH1/TH2 pattern of cytokines. J Allergy Clin Immunol. 1998 Dec; 102(6 Pt 1):953-60.

344. Bernstein JM, Ballow M, Rich G, Allen C, Swanson M, Dmochowski J. Lymphocyte subpopulations and cytokines in nasal polyps: is there a local immune system in the nasal polyp? Otolaryngology--Head and Neck Surgery. 2004 May; 130(5):526-35.

345. Cho KS, Kim CS, Lee HS, Seo SK, Park HY, Roh HJ. Role of interferon-gamma-producing t cells in the pathogenesis of chronic rhinosinusitis with nasal polyps associated with staphylococcal superantigen. J Otolaryngol Head Neck Surg. 2010 Oct; 39(5):600-5.

346. Maloy KJ, Powrie F. Regulatory T cells in the control of immune pathology. Nature Immunology. 2001 Sep; 2(9):816-22.

347. Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J, et al. Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. Lancet. 2004 Feb 21; 363(9409):608-15.

348. Karagiannidis C, Akdis M, Holopainen P, Woolley NJ, Hense G, Rückert B, et al. Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. J Allergy Clin Immunol. 2004 Dec; 114(6):1425-33.

349. Stelmach I, Jerzynska J, Kuna P. A randomized, double-blind trial of the effect of glucocorticoid, antileukotriene and beta-agonist treatment on IL-10 serum levels in children with asthma. Clinical and Experimental Allergy : Journal of the British Society for Allergy and Clinical Immunology. 2002 Feb; 32(2):264-9.

350. Thunberg S, Gafvelin G, Nord M, Gronneberg R, Grunewald J, Eklund A, et al. Allergen provocation increases TH2-cytokines and FOXP3 expression in the asthmatic lung. Allergy. 2010 Mar; 65(3):311-8.

351. Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of CD4+ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. J Allergy Clin Immunol. 1993 Aug; 92(2):313-24.

352. Tulic MK, Christodoulopoulos P, Fiset PO, Vaillancourt P, Lavigne F, Marshall JD, et al. Local induction of a specific Th1 immune response by allergen linked immunostimulatory DNA in the nasal explants of ragweed-allergic subjects. Allergology International. 2009 Dec; 58(4):565-72.

353. Provoost S, Maes T, van Durme YM, Gevaert P, Bachert C, Schmidt-Weber CB, et al. Decreased FOXP3 protein expression in patients with asthma. Allergy. 2009 Oct; 64(10):1539-46.

354. Hammad H, Lambrecht BN. Dendritic cells and epithelial cells: linking innate and adaptive immunity in asthma. Nature Reviews Immunology. 2008 Mar; 8(3):193-204.

355. Enk AH, Saloga J, Becker D, Mohamadzadeh M, Knop J. Induction of hapten-specific tolerance by interleukin 10 in vivo. J Exp Med. 1994 Apr 1; 179(4):1397-402.

356. Muller U, Akdis CA, Fricker M, Akdis M, Blesken T, Bettens F, et al. Successful immunotherapy with T-cell epitope peptides of bee venom

phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. J Allergy Clin Immunol. 1998 Jun; 101(6 Pt 1):747-54.

357. O'Garra A, Barrat FJ, Castro AG, Vicari A, Hawrylowicz C. Strategies for use of IL-10 or its antagonists in human disease. Immunological Reviews. 2008 Jun; 223:114-31.

358. Kearley J, Robinson DS, Lloyd CM. CD4+CD25+ regulatory T cells reverse established allergic airway inflammation and prevent airway remodeling. J Allergy Clin Immunol. 2008 Sep; 122(3):617-24 e6.

359. Dieckmann D, Bruett CH, Ploettner H, Lutz MB, Schuler G. Human CD4(+)CD25(+) regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells [corrected]. The Journal of Experimental Medicine. 2002 Jul 15; 196(2):247-53.

360. Grimbaldeston MA, Nakae S, Kalesnikoff J, Tsai M, Galli SJ. Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. Nature Immunology. 2007 Oct; 8(10):1095-104.

361. Blair PA, Norena LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, et al. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. Immunity. 2010 Jan 29; 32(1):129-40.

362. Shi J, Fan Y, Xu R, Zuo K, Cheng L, Xu G, et al. Characterizing T-cell phenotypes in nasal polyposis in Chinese patients. Journal of

investigational allergology & Clinical Immunology : official organ of the International Association of Asthmology. 2009; 19(4):276-82.

363. Min YG, Lee CH, Rhee CS, Hong SK, Kwon SH. Increased expression of IL-4, IL-5, IFN-gamma, IL-6, IL-8, and TGF-beta mRNAs in maxillary mucosa of patients with chronic sinusitis. American Journal of Rhinology. 1999 Sep-Oct; 13(5):339-43.

364. Chen YS, Arab SF, Westhofen M, Lorenzen J. Expression of interleukin-5, interleukin-8, and interleukin-10 mRNA in the osteomeatal complex in nasal polyposis. American Journal of Rhinology. 2005 Mar-Apr; 19(2):117-23.

365. Laffon A, Garcia-Vicuna R, Humbria A, Postigo AA, Corbi AL, de Landazuri MO, et al. Upregulated expression and function of VLA-4 fibronectin receptors on human activated T cells in rheumatoid arthritis. The Journal of Clinical Investigation. 1991 Aug; 88(2):546-52.

366. Fuss IJ, Neurath M, Boirivant M, Klein JS, de la Motte C, Strong SA, et al. Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. Journal of Immunology. 1996 Aug 1; 157(3):1261-70.

367. Marazuela M, Postigo AA, Acevedo A, Diaz-Gonzalez F, Sanchez-Madrid F, de Landazuri MO. Adhesion molecules from the LFA-1/ICAM-1,3 and VLA-4/VCAM-1 pathways on T lymphocytes and vascular endothelium in Graves' and Hashimoto's thyroid glands. European Journal of Immunology. 1994 Oct; 24(10):2483-90.

368. Li H, Llera A, Malchiodi EL, Mariuzza RA. The structural basis of T cell activation by superantigens. Annual Review of Immunology. 1999; 17:435-66.

369. Proft T, Fraser JD. Bacterial superantigens. Clinical and Experimental Immunology. 2003 Sep; 133(3):299-306.

370. Patou J, Gevaert P, Van Zele T, Holtappels G, van Cauwenberge P, Bachert C. Staphylococcus aureus enterotoxin B, protein A, and lipoteichoic acid stimulations in nasal polyps. J Allergy Clin Immunol. 2008 Jan; 121(1):110-5.

371. Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. Nature. 2008 Jul 24; 454(7203):445-54.

372. Bachert C, Wagenmann M, Rudack C, Hopken K, Hillebrandt M, Wang D, et al. The role of cytokines in infectious sinusitis and nasal polyposis. Allergy. 1998; 53(1):2-13.

373. Opal SM, DePalo VA. Anti-inflammatory cytokines. Chest. 2000 Apr; 117(4):1162-72.

374. Walker C, Virchow JC, Jr., Bruijnzeel PL, Blaser K. T cell subsets and their soluble products regulate eosinophilia in allergic and nonallergic asthma. Journal of Immunology. 1991 Mar 15; 146(6):1829-35.

375. Schleimer RP, Derse CP, Friedman B, Gillis S, Plaut M, Lichtenstein LM, et al. Regulation of human basophil mediator release by cytokines. I. Interaction with antiinflammatory steroids. Journal of Immunology. 1989 Aug 15; 143(4):1310-7.

376. Huehn J, Siegmund K, Lehmann JC, Siewert C, Haubold U, Feuerer M, et al. Developmental stage, phenotype, and migration distinguish

naive- and effector/memory-like CD4+ regulatory T cells. The Journal of Experimental Medicine. 2004 Feb 2; 199(3):303-13.

377. Rotzschke O, Borsellino G, Battistini L, Falk K, Kleinewietfeld M. In vivo-activated CD103+ Foxp3+ Tregs: of men and mice. Blood. 2009 Feb 26; 113(9):2119-20; author reply 20.

378. Van Bruaene N, Perez-Novo CA, Basinski TM, Van Zele T, Holtappels G, De Ruyck N, et al. T-cell regulation in chronic paranasal sinus disease. J Allergy Clin Immunol. 2008 Jun; 121(6):1435-41, 41 e1-3.

379. Li HB, Cai KM, Liu Z, Xia JH, Zhang Y, Xu R, et al. Foxp3+ T regulatory cells (Tregs) are increased in nasal polyps (NP) after treatment with intranasal steroid. Clinical Immunology. 2008 Dec; 129(3):394-400.

380. Dutsch-Wicherek M, Tomaszewska R, Lazar A, Strek P, Wicherek L, Kijowski J, et al. The presence of B7-H4+ macrophages and CD25+CD4+ and FOXP3+ regulatory T cells in the microenvironment of nasal polyps - a preliminary report. Folia histochemica et cytobiologica / Polish Academy of Sciences, Polish Histochemical and Cytochemical Society. 2010 Dec; 48(4):611-7.

381. Eastaff-Leung N, Mabarrack N, Barbour A, Cummins A, Barry S. Foxp3+ regulatory T cells, Th17 effector cells, and cytokine environment in inflammatory bowel disease. Journal of Clinical Immunology. 2010 Jan; 30(1):80-9.

382. Nistala K, Moncrieffe H, Newton KR, Varsani H, Hunter P, Wedderburn LR. Interleukin-17-producing T cells are enriched in the joints of

children with arthritis, but have a reciprocal relationship to regulatory T cell numbers. Arthritis and Rheumatism. 2008 Mar; 58(3):875-87.

383. Zhang N, Holtappels G, Gevaert P, Patou J, Dhaliwal B, Gould H, et al. Mucosal tissue polyclonal IgE is functional in response to allergen and SEB. Allergy. 2011 Jan; 66(1):141-8.

384. Keith PK, Conway M, Evans S, Wong DA, Jordana G, Pengelly D, et al. Nasal polyps: effects of seasonal allergen exposure. J Allergy Clin Immunol. 1994 Mar; 93(3):567-74.

385. Sanchez-Segura A, Brieva JA, Rodriguez C. Regulation of immunoglobulin secretion by plasma cells infiltrating nasal polyps. The Laryngoscope. 2000 Jul; 110(7):1183-8.

386. Galli SJ, Grimbaldeston M, Tsai M. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. Nature Reviews Immunology. 2008 Jun; 8(6):478-86.

387. Mazzoni A, Siraganian RP, Leifer CA, Segal DM. Dendritic cell modulation by mast cells controls the Th1/Th2 balance in responding T cells. Journal of Immunology. 2006 Sep 15; 177(6):3577-81.

388. Piconese S, Gri G, Tripodo C, Musio S, Gorzanelli A, Frossi B, et al. Mast cells counteract regulatory T-cell suppression through interleukin-6 and OX40/OX40L axis toward Th17-cell differentiation. Blood. 2009 Sep 24; 114(13):2639-48.

389. Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. Nature Immunology. 2005 Feb; 6(2):135-42.

390. Nie X, Cai G, Zhang W, Wang H, Wu B, Li Q, et al. Lipopolysaccharide mediated mast cells induce IL-10 producing regulatory T Cells through the ICOSL/ICOS axis. Clinical Immunology. 2011 Nov 20.

391. Powe DG, Jagger C, Kleinjan A, Carney AS, Jenkins D, Jones NS. 'Entopy': localized mucosal allergic disease in the absence of systemic responses for atopy. Clinical and Experimental Allergy : Journal of the British Society for Allergy and Clinical Immunology. 2003 Oct; 33(10):1374-9.

392. Forester JP, Calabria CW. Local production of IgE in the respiratory mucosa and the concept of entopy: does allergy exist in nonallergic rhinitis? Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2010 Oct; 105(4):249-55; quiz 56-8.

APPENDICES

Appendix 1 – Patient Information and Consent Forms

RANDALL
division of cell and molecular biophysics

3rd Floor
New Hunt's House
Guy's Campus
London SE1 1UL

Professor Hannah Gould
Telephone: 020 7848 6434
Fax: 020 7848 6435
Email: hannah.gould@kcl.ac.uk

KING'S College LONDON
University of London

PATIENT INFORMATION SHEET

TITLE OF PROJECT: Functional Analysis of Cell Populations Involved in Local Allergic Responses.

LAY TITLE OF PROJECT: Why some people develop nasal allergies to pollen, house dust mite and pets.

PRINCIPAL INVESTIGATORS: Prof. Hannah Gould, Dr. Pooja Takhar, Prof. Chris Corrigan, Dr. Alexander Faith, Ms Effy Chevretton, Mr. David Roberts, Dr. Narinderpal Singh

ETHICS COMMITTEE CODE NUMBER: 01/09/12

VERSION: 06 of 24/01/07

Introduction

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

Approximately half of all people make a special antibody called IgE to a variety of inhaled allergens such as grass pollen, housedust mite or cat fur. Nevertheless, only a very few of these patients then develop subsequent allergic disease such as hayfever or cat allergy. We want to try and understand why only some subjects are susceptible to developing hayfever. This might give us clues as to how to prevent its development. We think that one possibility is that some subjects make extra IgE antibody within their noses, which leads them to develop rhinitis. This may be because these allergic people have increased numbers, or specialised types, of cells in their nose. We want to see whether or not this is true by characterising the different types of cells in the nose and looking at the corresponding synthesis of IgE in the nose of patients with hayfever and other forms of rhinitis.

Why have I been chosen?

You have been asked to take part in this study because you are suffering from chronic rhinitis (inflammation of the lining of the nose) usually caused by allergies, which has necessitated surgical removal of some of the swollen tissue (either enlarged turbinates or polyps inside your nose.

Do I have to take part?

It is entirely up to you to decide whether or not to take part. If you do decide to take part you will be given this information to keep and asked to sign a Consent Form. Whether or not you take part will in no way compromise your treatment at the Allergy clinic.

What will happen to me if I take part?

If you decide to take part, we will ask you to donate the tissue excised during your surgery for us to analyse the cells present and compare the cellular profile to how much IgE is produced. Only the routine amount of tissue will be excised, no excess tissue will be taken. We will also ask you to provide a blood sample prior to your surgery. This amounts to about a teacupful (100ml) of blood. This is a relatively small quantity of blood, the taking of which will be of negligible risk to you. Blood sampling is a standard procedure, which may cause discomfort and bruising. If you have a history of any blood disorders including anaemia, please tell the supervising doctor before the blood sample is taken.

What will happen to the tissue I donate?

Most of it will be mashed up to release cells for our IgE studies. A small piece will be sent to GlaxoSmithKline, a pharmaceutical company developing new drugs for asthma and hayfever, where it will be stored in sections. By agreeing to participate in this study, you will therefore be donating a small portion of your sample to GlaxoSmithKline who may wish to use the research results for commercial or intellectual property (i.e. patent) purposes. Although GlaxoSmithKline may profit from the information they gain, you as a donor will have no commercial gain from the results of the research. Although authorised officials from GlaxoSmithKline may be allowed access to certain of your clinical details, they will not know that the samples have come from you.

What are the possible benefits of taking part?

Helping with this study will be of no direct benefit to you. The information that we get from the study may help with are ongoing research into the improvement of treatment and its application to a wider range of patients with polyps, hayfever and other forms of allergic rhinitis.

What if something goes wrong?

It is very unlikely that anything will go wrong. You should know, however, that if you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you will have grounds for complaint through the normal National Health Service complaints mechanism.

Will my taking part in this study be kept confidential?

The data obtained from this study may be reviewed by KCL staff and auditors. Data obtained from the study may be archived, published in a scientific journal and presented at international meetings. In such cases your name will not be used and confidentiality will be maintained.

What will happen to the results of this research study?

The results obtained from this study will be published as one or more papers in a scientific journal. You are perfectly free to obtain a copy of these studies if you wish. As explained above, however, your name and identity will not be identified in any report or publication.

Who is organising and funding the research?

This research is being funded by Asthma UK, a charitable foundation which provides funding for research into better treatments for asthma and allergy, and a Grant-In-Aid from the Garnett Passe and Rodney Williams Memorial Foundation, a charitable organisation which promotes research in diseases of the ears, nose and throat, to investigate the role of a particular type of cell called the dendritic cell in the allergic response. None of your doctors will be paid for including you in this study.

RANDALL
division of cell and molecular biophysics

3rd Floor
New Hunt's House
Guy's Campus
London SE1 1UL

Professor Hannah Gould
Telephone: 020 7848 6434
Fax: 020 7848 6435
Email: [illegible]

KING'S College LONDON
University of London

LREC Study Number:
Patient Identification Number for this trial:

CONSENT FORM

Title of Project: Functional Analysis of Cell Populations Involved in Local Allergic Responses (Ethics Committee Code Number: 01/09/12). Version 06 of 24/01/07

Name of Research (Delete as appropriate):

Professor Hannah Gould
Dr. Pooja Takhar
Prof. Chris Corrigan
Dr. Alexander Faith
Ms Elfy Chevetton
Mr. David Roberts
Dr. Narinderpal Singh

ENT DEPARTMENT
GUY'S HOSPITAL

Please initial box

1. I confirm that I have read and understand the information sheet (ersion 06 of 24/01/07) for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of any of my medical notes may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I agree to take part in the above study. ☐

Name of Patient	Date	Signature
Name of Person taking consent (if different from researcher)	Date	Signature
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

Appendix 2 – Raw data

Experiment 1 (NOT USED - ALK DILUENT)

Atopic (Cat)

Experiment: Cat allergen with ALK diluent

Conducted on day 6

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
2830.6	9.5	18.8	458.0	6.5	10.4	Control	
3232.6	11.3	21.3	198.7	4.6	7.7	Control	
2097.3	11.6	12.9	185.3	5.0	8.8	0.1	Cat + ALK
2463.2	12.2	14.9	315.2	6.0	11.0	0.1	
3103.1	10.1	19.0	252.7	4.3	**6.9**	0.01	
2665.8	10.3	20.1	178.0	4.4	4.5	0.01	
2033.2	7.7	17.9	265.7	3.3		0.001	

Experiment 2 (NOT USED - ALK DILUENT)

Atopic (Grass)

Experiment: Grass allergen with ALK diluent

Repeated with negative allergen (Cat)

Conducted on day 6

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
1314.5	61.5	14.4	68.3	4.7	22.2	Control	
1642.0	48.1	22.3	111.5	8.6	23.3	Control	
151.7	4.6	4.6	21.8	3.5	19.1	0.1	Grass + ALK
	4.0	5.6	8.6	4.6	12.3	0.1	
	6.5	5.9	68.6	3.3	**24.8**	0.01	
17.6	5.2	5.0	57.4	3.2	13.7	0.01	
79.9	3.9	5.9	132.0	3.8	20.5	0.001	
1142.4	9.6	9.0	141.5	4.5	6.5	0.001	
	3.5	23.5	4.0	1.6	4.1	0.1	Cat + ALK
	4.0	25.9	6.2	1.8	5.1	0.1	
45.7	7.8	56.4	12.1	1.7	**9.1**	0.01	
	9.5	52.5	13.7	1.6	1.4	0.01	
	6.7	41.1	11.8	1.5	8.3	0.001	
40.4	8.8	60.0	15.9	2.5	3.1	0.001	

Experiment 3

Atopic (Cat), Negative response to Grass

Experiment: Cat allergen with Hanks (HBSS) as diluent vs Cat + ALK diluent

Grass allergen with ALK diluent

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
180.4	8.4	118.7	44.8	6.6	5.6	Control	
531.4	10.1	134.7	59.1	17.8	29.0	Control	
	15.5	788.2	44.4	24.7	2.6	0.1	Cat + HBSS
375.2	15.2	778.5	32.8	25.7	2.2	0.1	
119.6	18.7	722.7	34.6	17.0		0.01	
92.5	18.6	676.5	40.5	19.0	4.8	0.01	
156.9	7.7	236.4	34.3	11.9	4.3	0.001	
	10.0	290.4	47.5	10.6	4.4	0.001	
327.9	8.4	93.5	36.6	13.3	6.6	0.0001	
191.9	7.0	101.6	48.2	15.5	6.0	0.0001	
	8.7	137.2	30.7	6.6	5.7	0.1	Cat + ALK
119.6	12.5	218.7	28.8	14.3	5.7	0.1	
269.3	15.7	489.4	44.4	9.9	3.7	0.01	
113.1	17.9	543.1	67.4	29.2	3.5	0.01	
	7.1	285.5	31.6	14.0	2.9	0.001	
	9.2	397.0	47.7	22.7	4.9	0.001	
	5.2	118.7	39.2	10.7	3.1	0.0001	
312.0	5.6	94.3	49.7	15.1	3.5	0.0001	
208.9	12.4	34.2	27.8	9.6	4.5	0.1	Grass + ALK
364.8	6.6	19.5	18.7	14.2	3.9	0.1	
966.7	13.4	37.2	35.9	40.6	12.4	0.01	
437.8	11.3	96.1	47.3	19.0	8.5	0.01	
231.1	3.3	35.3	39.4	6.6	4.4	0.001	
285.4	4.0	39.5	35.3	14.0	2.7	0.001	
132.4	3.0	40.5	35.9	12.9	4.9	0.0001	
	2.6	51.6	34.8	8.7	4.5	0.0001	

Experiment 4

Non-atopic, Negative response to all allergens

Experiment: Cat allergen with Hanks (HBSS) as diluent vs Cat + ALK diluent

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
783.5	21.4	79.3	37.6	37.5	2.6	Control	
280.0	18.7	93.9	35.0	24.7	2.2	Control	
448.2	28.0	547.6	28.0	25.9		0.1	Cat + HBSS
92.5	19.6	506.0	29.3	10.9		0.01	
92.5	19.8	361.9	31.5	12.4	1.8	0.001	
	13.6	120.9	32.5	7.5	2.9	0.0001	
879.9	14.4	163.9	20.6	16.6	9.9	0.1	Cat + ALK
531.4	42.4	501.8	35.3	33.5	5.2	0.01	
	19.8	248.9	27.5	12.2	3.4	0.001	
253.0	15.5	109.3	31.6	17.0	6.6	0.0001	

Experiment 5

Non atopic, Negative response to cat, grass

Experiment: Cat allergen with Hanks (HBSS) as diluent vs Cat + ALK diluent

Grass allergen with ALK diluent

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
741.1	22.3	67.7	22.4	26.2	8.3	Control	
593.9	16.5	49.3	20.3	32.9	5.5	Control	
630.5	31.3	335.5	23.9	25.7	9.4	0.1	Cat + HBSS
186.2	24.6	295.4	24.5	20.9	4.6	0.01	
751.7	26.4	287.9	21.5	27.1	8.8	0.001	
463.8	19.4	113.9	62.7	14.5	18.1	0.0001	
437.8	23.3	118.7	5.0	15.5	4.3	0.1	Cat + ALK
630.5	27.4	285.5	10.5	29.2	7.3	0.01	
3202.2	10.3	154.7	59.4	17.4	17.5	0.001	
406.6	13.2	111.4	23.3	18.8	3.4	0.0001	
236.6	13.4	17.2	6.3	5.7	30.7	0.1	Grass + ALK
106.4	11.6	39.7	13.4	11.6	4.5	0.01	
541.8	10.9	39.9	17.3	11.6	5.4	0.001	
280.0	12.3	52.6	22.0	13.8	2.6	0.0001	

Experiment 6

Atopic, Positive response to cat allergen

Experiment: Cat allergen with Hanks (HBSS) as diluent + anti IL10/ control

Repeated with negative antigen (grass + ALK diluent)

Conducted on day 2 and day 6

IFN-g	TNF-a	IL-10	IL-5	IL-4	IL-2		
pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml		
175.3	13.7	44.2	10.0	4.4	13.1	Control	
117.5	12.0	42.2	14.1	4.1	10.5	Control	
36.7	44.0	549.9	181.2	8.0	8.8	0.1	Pos antigen (Cat + HBSS) (DAY 2)
32.4	28.8	348.8	135.1	6.2	7.8	0.01	
48.7	17.7	259.7	179.7	5.6	6.8	0.001	
60.1	9.0	73.4	155.7	5.5	14.0	0.0001	
78.3	84.1	5.7	572.9	15.7	30.0	0.1	Pos antigen (Cat + HBSS) + anti-IL10 (DAY 2)
58.3	66.7	4.6	491.7	9.6	16.5	0.01	
49.6	36.5	4.4	487.1	9.5	22.7	0.001	
28.1	20.1	3.9	172.0	4.6	7.0	0.0001	
33.3	28.0	30.0	149.8	5.6	5.1	0.1	Pos antigen (Cat + HBSS) + control (DAY 2)
35.8	29.1	39.5	106.4	4.5		0.01	
	21.5	28.7	121.9	4.1	4.4	0.001	
29.0	8.8	12.9	129.4	3.7	3.0	0.0001	
	12.7	16.7	11.9	1.7	2.7	0.1	Neg antigen (grass + ALK) (DAY 2)
34.1	8.5	23.1	7.5			0.01	
54.8	6.0	4.8	7.7	1.7	5.3	0.001	
30.7	7.4	26.8	13.3		11.9	0.0001	
	24.1	2.8	9.2	1.1		0.1	Neg antigen (grass + ALK) + anti IL 10 (Day2)
	15.9	3.5	10.0		1.7	0.01	
	12.3	3.3	11.6	1.6	6.4	0.001	
29.0	14.4	3.9	16.8	2.2	5.6	0.0001	
	10.9	3.6	8.1	1.2		0.1	Neg antigen (grass + ALK) + control (DAY 2)
41.0	6.4	3.4	8.4	1.7	4.7	0.01	
39.3	6.1	4.4	6.8		3.8	0.001	
22.0	6.5	5.0	8.4		4.4	0.0001	
	6.2	17.9	30.0		6.7	Control	
	7.9	20.7	11.2	1.6	7.0	Control	
	11.9	112.7	348.2	3.8		0.1	Pos antigen (Cat + Hanks) DAY 6
22.0	11.0	89.9	451.7	2.6	7.0	0.01	
	8.8	56.1	163.3	2.5	2.5	0.001	
	4.8	18.0	201.3	2.3		0.0001	
20.2	13.9	3.1	1448.2	7.8	3.2	0.1	Pos antigen (Cat + Hanks) + anti-IL10 DAY 6

26.4	10.5	4.2	1395.6	7.1		0.01	
	7.3		996.6	2.0		0.001	
78.3	6.6	1.9	510.8	4.3	2.7	0.0001	
	12.6	122.9	237.1	4.7		0.1	Pos antigen (Cat + Hanks) + control DAY 6
	11.1	90.7	371.4	4.2	2.0	0.01	
	8.8	63.6	378.3	3.1	4.0	0.001	
	4.0	17.6	258.1	1.9	6.8	0.0001	
	5.5	5.1	13.0			0.1	Neg antigen (grass + ALK) DAY 6
	5.1	11.2	11.2	1.8		0.01	
61.9	7.0	10.7	10.4	2.3	3.4	0.001	
37.5	6.2	14.3	8.1			0.0001	
21.1	6.8	1.2	15.7	1.3	12.5	0.1	Neg antigen (grass + ALK) + anti IL 10 DAY 6
	6.6	4.6	10.2	2.1	2.3	0.01	
19.3	6.3	3.1	23.7			0.001	
	5.5	1.5	33.0			0.0001	
	7.8	3.6	12.1			0.1	Neg antigen (grass + ALK) + control DAY 6
	11.3	11.0	9.7	3.2	3.8	0.01	
26.4	3.9	6.5	37.5		5.3	0.001	
	2.8	2.5	7.9			0.0001	

Experiment 7

Atopic, Positive response to HDM allergen

Experiment: HDM allergen with Hanks (HBSS) as diluent + anti IL10/ control

Repeated with negative antigen (Cat + Hanks diluent)

Conducted on day 2 and day 6

IFN-g	TNF-a	IL-10	IL-5	IL-4	IL-2		
pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml		
84.0	8.9	102.3	4.8	1.9	16.4	Control	Day 2
144.1	9.6	60.6	6.1		11.3	Control	
131.0	7.2	112.3	8.0	2.9	8.4	0.1	Pos antigen (HDM + Hanks)
	6.8	63.8	6.7			0.01	
252.3	6.9	64.7	5.0	1.4	19.7	0.001	
367.0	9.4	65.0	8.7	2.7	38.5	0.0001	
233.3	23.0	7.5	21.5	2.9	19.1	0.1	Pos antigen (HDM + Hanks) + anti IL10
441.6	22.3	4.6	10.2	1.4	21.7	0.01	
215.0	12.5	2.5	7.6	1.6	8.1	0.001	
113.1	16.5	2.7	6.7	2.0	7.7	0.0001	
79.3	9.8	28.5	7.5	2.3	9.2	0.1	Pos antigen (HDM + Hanks) + control Ab
158.9	8.6	18.0	9.4	2.6	19.4	0.01	
60.8	7.2	13.4	4.8	1.8	18.4	0.001	
60.8	10.7	19.4	4.5		53.1	0.0001	
149.4	18.3	127.4	4.9	1.9	10.1	0.1	Neg antigen (Cat + Hanks)
79.3	18.1	494.4	5.7	3.6	11.4	0.01	
141.4	18.4	406.4	8.0	3.5	7.7	0.001	
100.8	11.8	164.6	4.7	2.4	13.7	0.0001	
487.9	14.3	47.8	8.4	2.4	14.4	Control	Day 6
203.0	8.1	43.3	7.0	2.8	14.5	Control	
751.4	9.4	40.1	18.2		38.9	0.1	Pos antigen (HDM + Hanks) Day 6
263.6	7.4	21.8	6.5	1.8	8.4	0.01	
571.2	4.8	20.5	6.3	2.8	19.0	0.001	
140.1	6.6	25.8	4.3	2.3	15.1	0.0001	
417.3	12.8	4.4	13.2		52.6	0.1	Pos antigen (HDM + Hanks) + anti IL10 Day 6
288.5	10.6	4.0	8.8		8.5	0.01	
216.5	8.6	4.3	18.2	2.5	7.3	0.001	
880.9	11.6	3.3	9.5	3.8	75.6	0.0001	
641.4	15.0	19.0	10.4	2.6	15.1	0.1	Pos antigen (HDM + Hanks) + control Ab Day 6
228.7	6.8	6.1	6.4		4.4	0.01	
312.6	5.5	6.1	4.3		9.6	0.001	
206.0	6.6	9.5	4.7		6.1	0.0001	
102.0	7.7	82.0	9.5	2.9	8.3	0.1	Neg antigen (Cat + Hanks) Day 6
132.3	11.2	118.1	8.7		5.5	0.01	
367.0	19.2	116.1	6.4	1.6	15.1	0.001	

227.2	13.4	49.1	5.7	3.3		0.0001

Experiment 8

Non atopic

Experiment: HDM allergen with Hanks (HBSS) as diluent + anti IL10/ control

Conducted on day 2 and day 6

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
	13.7	86.3	158.7	3.5	53.1	Control	
	12.2	53.1	142.8	2.9	72.0	Control	
63.1	15.4	197.1	271.9	3.3	58.2	0.1	Neg antigen (HDM + Hanks) Day 2
	15.3	67.3	119.1	2.5	109.0	0.01	
	11.0	77.9	156.6	1.9	70.2	0.001	
113.1	12.7	90.8	99.3	2.8	15.7	0.0001	
1030.1	37.6	6.2	329.4	3.8	114.0	0.1	Neg antigen (HDM + Hanks) + anti IL10 Day 2
247.5	18.5	4.9	236.3		104.2	0.01	
	13.4	3.7	119.1	2.9	29.6	0.001	
	19.6	3.3	176.5	1.8	8.9	0.0001	
58.5	15.6	34.2	121.7	2.0	44.3	0.1	Neg antigen (HDM + Hanks) + control Ab Day 2
	24.0	23.1	164.4	3.9	182.5	0.01	
	16.1	17.3	240.7	2.9	151.8	0.001	
65.4	13.5	24.5	183.0	4.1	128.4	0.0001	
79.3	14.3	54.3	191.4	1.9	40.6	Control	
60.8	11.7	49.9	278.5	4.1	33.5	Control	
91.1	18.3	101.4	279.8	3.0	18.0	0.1	Neg antigen (HDM + Hanks) Day 6
93.5	10.9	36.4	194.9	2.0	22.0	0.01	
	14.5	38.5	253.4	1.4	33.4	0.001	
708.3	31.6	30.0	266.9	3.8	70.5	0.0001	
197.1	14.3	5.0	349.2	1.4	6.3	0.1	Neg antigen (HDM + Hanks) + anti IL10 Day 6
729.7	17.0	2.8	349.2	3.6	16.2	0.01	
501.0	16.0	2.3	461.7		8.7	0.001	
854.0	21.1	4.2	726.0	1.7	10.8	0.0001	
161.7	17.0	82.0	274.5	2.9	19.7	0.1	Neg antigen (HDM + Hanks) + control Ab Day 6
81.6	19.7	15.6	309.3	4.1	68.8	0.01	
352.1	24.2	24.5	443.4	2.1	16.9	0.001	
66.5	17.2	126.9	261.9	2.0	20.7	0.0001	

Experiment 9 (NOT USED - NON-RESPONDER)

Non-Atopic

Experiment: Cat allergen with Hanks (HBSS) as diluent

All repeated with negative antigen (HDM)

Conducted on day 6

No responses

IFN-g	TNF-a	IL-10	IL-5	IL-4	IL-2		
pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml		
24.2	3.4	2.6	4.9	3.5	15.9	Control	Control
23.6	3.1	2.5	4.2	3.1	26.1	Control	Control
36.5	3.2	3.4	2.6	3.0	20.8	0.1	Neg antigen (HDM) 0.1
23.0	4.6	2.8	4.3	3.0	23.7	0.001	Neg antigen (HDM) 0.001
36.5	3.0	3.1	4.3	3.0	14.8	0.1	Neg antigen (Cat) 0.1
32.4	2.6	3.1	3.1	4.4	21.6	0.001	Neg antigen (Cat) 0.001

Experiment 10 (NOT USED - NON-RESPONDER)

Non atopic

Experiment: Cat allergen with Hanks (HBSS) as diluent

Conducted on day 6

No significant response noted in entire experiment

Note that this patient had very small amount of tissue with small number of cells (4 X 10^6 total)

IFN-g	TNF-a	IL-10	IL-5	IL-4	IL-2		
pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml		
201.5	6.8	6.3	3.5	3.4	11.0	Control	Control
218.0	16.0	9.8	5.6	9.2	22.7	Control	Control
56.3	5.2	3.3	3.4	3.2	10.1	0.1	Neg antigen (Cat + Hanks) 0.1
	4.7	4.8	2.8	3.1	9.2	0.001	0.001

Experiment 11

Non- Atopic

Experiment: grass allergen with Hanks (HBSS) as diluent

Conducted on day 2 and 6

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
43.7	45.4	319.5	32.7	4.2	25.3		Control
38.6	39.1	296.2	32.0	4.4	26.9		Control
35.1	35.5	265.5	28.2	3.5	25.3	0.1	Neg antigen 0.1 Day 2
54.0	33.1	248.2	32.7	5.5	17.2	0.001	Neg antigen 0.001 Day 2
38.6	18.1	188.7	30.3	4.2	23.7		Control
37.2	15.2	184.0	12.6	3.2	20.6		Control
32.4	16.7	149.5	21.5	2.6	16.9	0.1	Neg antigen 0.1 Day 6
4.9	11.6	123.0	14.0	2.6		0.001	Neg antigen 0.001 Day 6

Experiment 12

Atopic (Grass)

Experiment: Grass allergen with Hanks (HBSS) as diluent

All repeated with negative antigen (cat)

Conducted on day 2 and day 6

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
597.1	15.3	425.5	651.4	6.4	23.5	Control	Control
426.6	16.3	361.7	522.2	4.1	24.8	Control	Control
90.3	11.0	398.7	171.8	7.8	34.0	0.1	Pos antigen (Grass) Day 2 0.1
481.2	13.7	297.8	204.0	6.1	27.1	0.001	Pos antigen (Grass) Day 2 0.001
76.9	14.0	454.2	36.5	7.6	25.7	0.1	Neg antigen (Cat) Day 2 0.1
233.2	25.9	702.7	53.9	5.2	30.1	0.001	Neg antigen (Cat) Day 2 0.001
354.7	20.0	73.7	34.2	24.4	50.9	Control	Control
505.7	13.1	70.0	35.9	3.5	27.5	Control	Control
137.9	7.0	72.4	115.8	5.6	27.9	0.1	Pos antigen (Grass) Day 6 0.1
216.5	7.5	70.9	65.9	6.3	40.3	0.001	Pos antigen (Grass) Day 6 0.001
66.9	12.1	91.0	13.4	2.4		0.1	Neg antigen (Cat) Day 6 0.1
91.4	18.8	92.6	27.0	3.8	24.8	0.001	Neg antigen (Cat) Day 6 0.001

Experiment 13

Atopic (Grass + HDM)

Experiment: Grass allergen with Hanks (HBSS) as diluent

Repeated with negative antigen (cat) and HDM allergen (positive)

Conducted on day 2

IFN-g	TNF-a	IL-10	IL-5	IL-4	IL-2		
pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml		
15.3	6.9	2.4	21.1	3.1	25.7		Control
35.7	14.7	5.5	36.5	3.3	29.2		Control
37.6	29.3	21.7	40.6	4.6	22.1	0.1	Pos antigen (Grass) Day 2 0.1
21.8	1.7				16.4	0.001	Pos antigen (Grass) Day 2 0.001
37.6	26.4	21.8	29.8	2.3	27.1	0.1	Pos antigen (HDM) Day 2 0.1
	10.1	5.0	29.0		24.4	0.001	Pos antigen (HDM) Day 2 0.001
	4.9		10.9		22.1	0.1	Neg antigen (Cat) Day 2 0.1
	5.9	2.4	14.1	3.9	26.6	0.001	Neg antigen (Cat) Day 2 0.001

Experiment 14

Atopic (Grass)

Experiment: Grass allergen with Hanks (HBSS) as diluent All repeated with negative antigen (cat)

Middle turbinate tissue compared with polyp tissue

Conducted on day 2

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
1608.4	37.5	411.9	88.3	3.1			Control
883.1	40.0	454.2	69.2	5.1	32.2		Control
610.9	43.8	979.3	75.7	3.2	25.7	0.1	Pos antigen (Grass) Day 2 0.1
535.2	39.6	679.7	53.4	4.7	16.4	0.001	Pos antigen (Grass) Day 2 0.001
2065.9	28.5	343.1	36.8	5.0	33.5	0.1	Neg antigen (Cat) Day 2 0.1
1458.5	33.2	328.2	40.8	5.5	24.8	0.001	Neg antigen (Cat) Day 2 0.001
106.4	37.8	228.0	7.3		26.6	0.1	MT Pos antigen (Grass) Day 2 0.1
19.7	14.0	102.4	4.9		17.4	0.001	MT Pos antigen (Grass) Day 2 0.001
	7.9	36.7	2.9	2.5	16.4	0.1	MT Neg antigen (Cat) Day 2 0.1
	8.0	68.8	22.6	4.0	19.3	0.001	MT Neg antigen (Cat) Day 2 0.001

Experiment 15

Atopic (Grass, HDM, Cat, Dog)

Experiment: Grass allergen with Hanks (HBSS) as diluent

All repeated with Cat and HDM antigen

Conducted on day 6

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
40.4	5.8	42.7	20.2	4.7	18.0		Control
36.8	4.2	33.7	11.2	1.7	16.9		Control
162.4	15.1	166.9	78.4	4.5	15.3	0.1	Pos antigen (Grass) 0.1
55.7	6.8	83.6	25.6	4.2	11.6	0.001	Pos antigen (Grass) 0.001
25.7	10.4	15.4	6.1	3.5	24.8	0.1	Pos antigen (Cat) 0.1
64.5	6.5	79.3	14.3	3.7	22.7	0.001	Pos antigen (Cat) 0.001
43.0	8.9	205.3	22.0	3.3	16.4	0.1	Pos antigen (HDM) 0.1
9.0	3.0	36.1	14.2	2.2		0.001	Pos antigen (HDM) 0.001

Experiment 16 (NOT USED - NON-RESPONDER)

Atopic (Grass, HDM)

Experiment: Grass allergen with Hanks (HBSS) as diluent

All repeated with Cat and HDM antigen

Conducted on day 6

IFN-g	TNF-a	IL-10	IL-5	IL-4	IL-2			
pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml			
	2.4	1.7	1.1	3.1		Control	Control	
5.7	3.5	1.5	2.3	2.0	3.5	Control	Control	
	5.0		3.7		1.9	0.1	Pos antigen (Grass)	0.1
	5.0	1.6	3.8	1.1	7.6	0.001	Pos antigen (Grass)	0.001
	3.3	1.3	1.1		3.8	0.1	Pos antigen (HDM)	0.1
	2.6	1.6	1.3			0.001	Pos antigen (HDM)	0.001
4.9	2.9	2.6	2.0	1.3	8.1	0.1	Neg antigen (Cat)	0.1
	3.8	1.4	1.6		1.9	0.001	Neg antigen (Cat)	0.001

Experiment 17

Atopic (Grass)

Experiment: Grass allergen with Hanks (HBSS) as diluent

Repeated using (negative) Cat and HDM antigen

Conducted on day 6

IFN-g	TNF-a	IL-10	IL-5	IL-4	IL-2		
pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml		
28.6		33.3	68.9		19.6	Control	Control
33.2		37.3	50.7		24.5	Control	Control
59.3		272.1	830.0			0.1	Pos antigen (Grass) 0.1
28.0		193.5	528.0			0.001	Pos antigen (Grass) 0.001
22.0		94.4				0.1	Neg antigen (cat) .1
51.6		180.7	63.9			0.001	Neg antigen (cat) .001
40.6		227.5	86.2			0.1	Neg antigen (HDM) .1
47.6	26.9	519.4	95.3		21.9	0.001	Neg antigen (HDM) .001

Experiment 18 (NOT USED - INFECTED)

Atopic (Grass)

Experiment: Grass allergen with Hanks (HBSS) as diluent

Repeated with negative antigen (cat, HDM)

Conducted on day 6

INFECTED - NOT PROCESSED

Experiment 19

Atopic (Grass, HDM)

Experiment: Grass allergen with Hanks (HBSS) as diluent

Repeated with addition of anti-IL10

Repeated using (positive) HDM and (negative) Cat antigen

Conducted on day 6

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
8.4	2.3	2.3	4.1	2.9		Control	Control
12.4	3.0	2.8	4.3	2.5	11.0	Control	Control
14.6	10.3	38.7	4.8	3.3	12.0	0.1	Pos antigen (Grass) 0.1
9.6	4.5	12.8	4.8	2.2		0.001	Pos antigen (Grass) 0.001
26.2	6.6	4.1	6.0	2.9	16.6	0.001	Pos antigen (Grass) 0.001+antiIL10
48.8	7.0	4.2	9.0	4.2	29.5	0.1	Pos antigen (Grass) 0.1 5X
33.4	5.5	4.5	10.5	3.5	26.5	0.001	Pos antigen (Grass) 0.001 5X
28.2	7.0	4.4	7.4	4.1	25.1	0.1	Pos antigen (HDM) 0.1
19.3	11.2	9.1	7.1	2.5	21.4	0.001	Pos antigen (HDM) 0.001
17.0	4.1	2.8	6.6	2.1	11.2	0.1	Pos antigen (HDM) 0.1 5X
11.8	3.1	2.5	6.6	2.2	11.2	0.001	Pos antigen (HDM) 0.001 5X
18.7	2.7	3.3	4.9	3.1	12.8	0.1	Neg antigen (Cat) 0.1
23.0	10.3	32.9	5.6	2.8	8.5	0.001	Neg antigen (Cat) 0.001
10.7	3.4	2.7	6.7	2.6	10.3	0.1	Neg antigen (Cat) 0.1 5X
95.8	8.1	3.5	11.4	3.3	20.5	0.001	Neg antigen (Cat) 0.001 5X

Experiment 20

Atopic (Grass, HDM, weak + Cat on RAST)

Experiment: Grass allergen with Hanks (HBSS) as diluent

Repeated with addition of anti-IL10 + Rat control Ab and + mouse control Ab

Repeated using (positive) HDM and (negative) Cat antigen

Conducted on day 6

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
1076.9	30.1	32.9	101.2	5.7	48.8		Control
1356.5	26.7	27.9	258.1	5.9	21.9		Control
110.0	25.1	180.4	151.5	5.2	16.0	0.1	Pos antigen (Grass) 0.1
350.3	30.7	178.7	222.5	6.8	23.3	0.1	Pos antigen (Grass) +Rat anticontrolAb 0.1
86.6	26.7	178.7	263.0	5.2	23.3	0.1	Pos antigen (Grass) +Mouse anticontrolAb 0.1
862.7	11.3	286.3	410.2	5.3	21.0	0.001	Pos antigen (Grass) 0.001
1213.4	9.5	260.3	335.8	4.6	6.1	0.001	Pos antigen (Grass) +Rat anticontrolAb 0.001
906.0	9.1	281.2	380.0	5.4	17.0	0.001	Pos antigen (Grass) +Mouse anticontrolAb 0.001
300.2	40.1	5.8	648.8	8.8	26.1	0.1	Pos antigen (Grass) 0.1 +anti IL10
1765.5	14.4	5.9	348.8	5.3	9.5	0.001	Pos antigen (Grass) 0.001+antiIL10
207.4	60.6	20.1	488.1	4.7	19.6	0.1	Pos antigen (Grass) 0.1 5X
213.3	28.7	10.0	302.7	5.5	10.3	0.001	Pos antigen (Grass) 0.001 5X
104.9	13.0	22.8	117.8	4.0	16.2	0.1	Pos antigen (HDM) 0.1
538.7	12.3	46.3	406.3	5.3	11.2	0.001	Pos antigen (HDM) 0.001
438.1	17.9	11.7	276.8	4.2	16.2	0.1	Pos antigen (HDM) 0.1 5X
792.6	23.9	56.9	317.3	4.6	16.2	0.001	Pos antigen (HDM) 0.001 5X
87.2	4.1	4.1	42.1	2.3	10.7	0.1	Neg antigen (Cat) 0.1
957.8	16.6	37.9	191.2	4.3	10.3	0.001	Neg antigen (Cat) 0.001
781.5	5.9	4.6	352.1	5.8	12.2	0.1	Neg antigen (Cat) 0.1 5X
466.1	23.2	65.4	196.5	3.5	12.0	0.001	Neg antigen (Cat) 0.001 5X

Experiment 21 (NOT USED - NON-RESPONDER)

Atopic (Grass)

Experiment: Grass allergen with Hanks (HBSS) as diluent

Repeated with addition of anti-IL10 + Rat control Ab and + mouse control Ab

Repeated using (negative) HDM and (negative) Cat antigen

Conducted on day 6

IFN-g	TNF-a	IL-10	IL-5	IL-4	IL-2		
pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml		
13.4	10.5	4.3	9.8	2.5	2.9	Control	Control
7.8	9.7	2.1	11.0	2.3	4.0	Control	Control
5.0	10.7	6.7	10.4	1.7	4.2	0.1	Pos antigen (Grass) 0.1
4.0	8.3	4.6	8.1	1.1	1.7	0.1	Pos antigen (Grass) +Rat anticontrolAb 0.1
4.0	12.4	5.7	10.3	1.7	4.6	0.1	Pos antigen (Grass) +Mouse anticontrolAb 0.1
2.9	6.7	4.6	9.2	2.4		0.001	Pos antigen (Grass) 0.001
	5.4	2.1	8.4		4.3	0.001	Pos antigen (Grass) +Rat anticontrolAb 0.001
3.6	6.9	3.8	8.4	1.9	2.1	0.001	Pos antigen (Grass) +Mouse anticontrolAb 0.001
4.0	9.1	1.8	8.8	1.7	2.7	0.1	Pos antigen (Grass) 0.1 +anti IL10
	9.8	4.2	8.2	2.5	4.5	0.001	Pos antigen (Grass) 0.001+antiIL10
4.3	6.6	1.4	8.4	1.5	2.7	0.1	Neg antigen (HDM) 0.1
4.5	12.2	2.0	8.9	2.0	2.3	0.001	Neg antigen (HDM) 0.001
	2.1		6.4		1.2	0.1	Neg antigen (Cat) 0.1
	6.1	1.6	9.8	1.6	2.7	0.001	Neg antigen (Cat) 0.001

Experiment 22 (NOT USED - NON-RESPONDER)

Atopic (Grass)

Experiment: Grass allergen with Hanks (HBSS) as diluent + anti IL 10 and + Rat control Ab and + mouse control Ab

Repeated using (negative) HDM and (negative) Cat antigen

Conducted on day 6

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
	2.9	1.2	15.2			Control	Control
	3.9	1.7	12.5			Control	Control
	10.0	6.0	13.4			0.1	Pos antigen (Grass) 0.1
	17.2	3.5	12.8			0.1	Pos antigen (Grass) +anti IL10 0.1
	14.0	7.5	13.8		2.1	0.1	Pos antigen (Grass) +Rat anticontrolAb 0.1
	15.0	9.6	14.9			0.1	Pos antigen (Grass) +Mouse anticontrolAb 0.1
	7.7	2.3	14.4			0.001	Pos antigen (Grass) 0.001
	17.0		14.4			0.001	Pos antigen (Grass) +anti IL10 0.001
1.9	11.0		12.1			0.001	Pos antigen (Grass) +Rat anticontrolAb 0.001
	11.7	2.2	12.6			0.001	Pos antigen (Grass) +Mouse anticontrolAb 0.001
	10.1	1.4	11.7			0.1	Neg antigen (HDM) 0.1
	5.7	1.7	13.8			0.001	Neg antigen (HDM) 0.001
	3.7	2.1	10.7			0.1	Neg antigen (Cat) 0.1
	6.1	1.4	13.4			0.001	Neg antigen (Cat) 0.001

Experiment 23

Non Atopic

Experiment: Grass allergen with Hanks (HBSS) as diluent + anti IL 10 and + Rat control Ab and + mouse control Ab

Repeated using (negative) HDM and (negative) Cat antigen

Conducted on day 6

IFN-g	TNF-a	IL-10	IL-5	IL-4	IL-2		
pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml		
	4.2	1.7	98.8			Control	Control
	3.7	1.8	73.0			Control	Control
3.4	25.2	18.5	81.0			0.1	Neg antigen (Grass) 0.1
3.4	35.7	5.9	82.4		5.4	0.1	Neg antigen (Grass) +anti IL10 0.1
	28.6	7.2	90.6	1.6		0.1	Neg antigen (Grass) +Rat anticontrolAb 0.1
	38.5	54.8	93.0			0.1	Neg antigen (Grass) +Mouse anticontrolAb 0.1
	7.9	18.7	54.0		5.7	0.001	Neg antigen (Grass) 0.001
	15.9	5.6	57.3		4.4	0.001	Neg antigen (Grass) +anti IL10 0.001
	7.9	12.4	55.9		6.3	0.001	Neg antigen (Grass) +Rat anticontrolAb 0.001
	11.5	51.5	66.4	1.7		0.001	Neg antigen (Grass) +Mouse anticontrolAb 0.001
	15.7	2.2	56.4	1.8		0.1	Neg antigen (HDM) 0.1
	26.1	140.6	80.3		4.7	0.001	Neg antigen (HDM) 0.001
	3.7	8.6	42.1		4.7	0.1	Neg antigen (Cat) 0.1
	4.4	2.5	71.1			0.001	Neg antigen (Cat) 0.001

Experiment 24

Atopic (Grass, Cat, Tree)

Experiment: Grass allergen with Hanks (HBSS) as diluent and + anti IL 10

Cat allergen with Hanks (HBSS) as diluent and + anti IL 10

(negative) HDM

T cells depleted using MACS column, control without depletion

Conducted on day 6

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
4.4	10.1	221.2	335.9	5.4	4.2	Control	Control
6.6	18.3	179.6	391.8	7.1	9.2	Control	Control
37.2	329.5	1460.1	140.9	4.8	2.7	0.1	Pos antigen (Grass) 0.1
60.6	765.8	9.9	264.2	5.5	3.7	0.1	Pos antigen (Grass) +anti IL10 0.1
15.3	20.0	285.6	223.7	3.6	4.0	0.1	Pos antigen (cat) 0.1
27.4	26.7	4.3	291.8	7.0	5.4	0.1	Pos antigen (cat) +anti IL10 0.1
21.1	209.1	1989.3	104.5	4.6	3.5	0.1	Neg antigen (HDM) 0.1
11.6	25.7	853.6	106.4	3.3	2.7	0.001	Pos antigen (Grass) 0.001
85.3	41.2	12.2	266.8	4.2	2.6	0.001	Pos antigen (Grass) +anti IL10 0.001
5.5	312.8	122.0	29.1	3.2	3.1	0.001	Pos antigen (cat) 0.001
6.7	602.0	5.0	36.2	2.8	3.4	0.001	Pos antigen (cat) +anti IL10 0.001
61.9	34.8	1105.6	256.5	5.1	4.6	0.001	Neg antigen (HDM) 0.001
11.4	131.2	394.4	41.9	2.7	2.4	0.1	Pos antigen (Grass) T depleted 0.1
5.7	24.5	482.1	24.5	3.4	3.2	0.001	Pos antigen (Grass) T depleted 0.001
38.9	736.7	2105.0	104.5	3.0	2.3	0.1	Pos antigen (Grass) control 0.1
23.8	45.7	1590.5	115.4	3.7	3.7	0.001	Pos antigen (Grass) control 0.001
4.2	11.1	70.9	4.5	3.7	3.1	0.1	Pos antigen (Cat) T depleted 0.1
4.7	141.4	45.8	5.9	3.3	2.3	0.001	Pos antigen (Cat) T depleted 0.001
8.4	21.5	484.3	62.9	4.1	2.3	0.1	Pos antigen (Cat) control 0.1
4.5	568.4	343.3	36.3	2.7	3.2	0.001	Pos antigen (Cat) control 0.001
4.0	140.0	457.9	28.0	3.1	2.3	0.1	Neg antigen (HDM) T depleted 0.1

5.8	22.6	457.9	12.4	3.0	2.3	0.001	Neg antigen (HDM) T depleted 0.001
20.5	695.3	2105.0	124.0	2.9	2.5	0.1	Neg antigen (HDM) control 0.1
13.5	55.9	2105.0	163.5	3.8	2.9	0.001	Neg antigen (HDM) control 0.001

Experiment 25

Non - Atopic

Experiment: Grass allergen with Hanks (HBSS) as diluent and + anti IL 10

Repeated with cat and HDM + anti IL 10

Conducted on day 6

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
41.7	45.1	82.2	361.3	5.2	23.4		Control
46.6	26.4	64.7	204.0	4.8	29.2		Control
31.5	28.4	362.4	159.2	5.9	12.6	0.1	Neg antigen (Grass) 0.1
108.0	21.2	8.0	880.3	5.4	31.9	0.1	Neg antigen (Grass) +anti IL10 0.1
15.8	19.8	222.9	49.3	5.9	13.3	0.1	Neg antigen (cat) 0.1
23.1	28.4	6.8	75.7	4.5	10.7	0.1	Neg antigen (cat) +anti IL10 0.1
94.9	17.5	216.4	178.3	3.4	8.5	0.1	Neg antigen (HDM) 0.1
321.0	53.2	7.6	1000.4	6.0	9.5	0.1	Neg antigen (HDM) +anti IL10 0.1
40.1	179.0	132.1	99.6	4.4	10.7	0.001	Neg antigen (Grass) 0.001
795.4	42.6	7.5	1204.3	3.0	18.8	0.001	Neg antigen (Grass) +anti IL10 0.001
16.9	25.6	136.7	103.0	5.0	12.6	0.001	Neg antigen (cat) 0.001
23.3	81.9	3.1	215.8	3.5	3.9	0.001	Neg antigen (cat) +anti IL10 0.001
96.9	26.2	556.6	138.1	4.6	8.8	0.001	Neg antigen (HDM) 0.001
371.9	36.9	8.5	902.8	6.6	20.4	0.001	Neg antigen (HDM) +anti IL10 0.001

Experiment 26

Atopic (Cat, tree)

Experiment: Cat allergen with Hanks (HBSS) as diluent and + anti IL 10

Grass and HDM

Conducted on day 6

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
18.9	7.9	3.4	11.1	4.4	12.6		Control
14.6	4.4	3.2	11.0	4.2	8.1		Control
28.5	6.8	4.3	7.6	7.0	9.5	0.1	Pos antigen (Cat) 0.1
23.9	7.1	3.4	7.1	6.9	9.9	0.1	Pos antigen (Cat) +anti IL10 0.1
16.9	13.5	3.1	10.8	6.3	9.1	0.1	Neg antigen (Grass) 0.1
25.3	8.7	5.3	12.1	5.0	12.0	0.1	Neg antigen (HDM) 0.1
78.5	18.7	126.6	24.4	3.4	16.2	0.001	Pos antigen (Cat) 0.001
27.6	29.5		32.6	4.3	9.7	0.001	Pos antigen (Cat) +anti IL10 0.001
16.6	21.8	2.9	14.8	4.4	11.3	0.001	Neg antigen (Grass) 0.001
10.6	27.9	300.8	16.6	4.6	9.1	0.001	Neg antigen (HDM) 0.001

Experiment 27

Atopic (Cat, HDM)

Experiment: HDM allergen with Hanks (HBSS) as diluent and + anti IL 10

Repeated with Cat and grass alone

Conducted on day 6

Round bottom plate used as flat bottom plates ran out

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
19.7	7.8	5.7	22.4	6.2	10.8		Control
19.7	8.2	3.2	23.5	7.2	14.7		Control
28.5	11.0	8.0	20.4	10.3	13.3	0.1	Pos antigen (HDM) 0.1
17.2	8.9	5.4	17.6	6.5	12.6	0.1	Pos antigen (HDM) +anti IL10 0.1
30.9	9.6	6.3	10.2	10.4	19.0	0.1	Pos antigen (cat) 0.1
27.9	7.8	5.0	7.7	6.7	11.1	0.1	Pos antigen (cat) +anti IL10 0.1
32.7	10.5	5.4	10.5	8.0	15.9	0.1	Neg antigen (Grass) 0.1
31.2	9.1	8.0	21.8	6.6	10.8	0.001	Pos antigen (HDM) 0.001
41.0	11.6	8.5	20.9	11.3	21.1	0.001	Pos antigen (HDM) +anti IL10 0.001
33.9	9.5	4.5	15.6	7.0	15.2	0.001	Pos antigen (cat) 0.001
33.9	9.4	5.2	17.6	8.2	10.8	0.001	Pos antigen (cat) +anti IL10 0.001
38.2	16.0	7.5	16.3	12.3	17.4	0.001	Neg antigen (Grass) 0.001

Experiment 28

Non - Atopic

Experiment: HDM allergen with Hanks (HBSS) as diluent and + anti IL 10

Repeated with cat and grass

T cells depleted using MACS column, control without depletion

Conducted on day 6

IFN-g pg/ml	TNF-a pg/ml	IL-10 pg/ml	IL-5 pg/ml	IL-4 pg/ml	IL-2 pg/ml		
54.2	50.5	306.1	47.0	6.2	9.9		Control
101.8	52.9	204.7	39.5	5.2	12.0		Control
26.8	52.4	304.8	37.9	3.2	9.7	0.1	Neg antigen (HDM) 0.1
116.6	47.5	6.9	83.2	4.2	9.7	0.1	Neg antigen (HDM) +anti IL10 0.1
27.1	20.3	95.3	22.4	5.3	11.6	0.1	Neg antigen (cat) 0.1
1036.6	30.4	4.7	35.6	5.0	12.3	0.1	Neg antigen (cat) +anti IL10 0.1
14.4	27.0	286.9	46.6	4.3	2.7	0.1	Neg antigen (Grass) 0.1
88.3	38.3	8.0	119.6	5.5	10.2	0.1	Neg antigen (Grass) +anti IL10 0.1
	37.1	231.6	38.9	2.9	13.0	0.001	Neg antigen (HDM) 0.001
93.0	35.8	7.1	67.0	4.4	10.5	0.001	Neg antigen (HDM) +anti IL10 0.001
11.5	25.6	172.7	34.2	5.7	11.4	0.001	Neg antigen (cat) 0.001
63.1	28.1	6.3	47.0	1.9	10.1	0.001	Neg antigen (cat) +anti IL10 0.001
50.7	38.7	277.2	57.9	6.1	6.9	0.001	Neg antigen (Grass) 0.001
1086.7	81.9	6.5	140.5	5.0	6.9	0.001	Neg antigen (Grass) +anti IL10 0.001
3.0	10.1	98.3	29.9	3.5	13.9		Control T dep
11.2	10.2	90.5	15.1	7.5	9.7		Control T dep
18.9	17.0	347.0	39.0	2.7	10.8	0.1	HDM 0.1 T dep
	8.3	39.5	5.0	3.3	6.5	0.001	HDM 0.001 T dep
14.9	21.5	201.3	28.9	5.0	10.2	0.1	Cat 0.1 T dep
8.4	12.5	128.8	12.0	2.1	9.4	0.001	Cat 0.001 T dep
13.2	11.3	65.9	34.5	3.1	12.5	0.1	Grass 0.1 T dep
36.3	33.1	241.7	41.7	6.6	14.1	0.001	Grass 0.001 T dep
13.2	10.1	97.0	17.3	3.4	8.5		Control Column alone
12.1	12.4	117.2	24.1	2.3	9.1		Control Column alone

25.1	18.4	459.4	36.2	4.4	4.2	0.1	HDM 0.1 Column alone
8.4	9.5	83.7	25.4	3.6	13.3	0.001	HDM 0.001 Column alone
6.2	18.3	323.8	27.6	3.7	6.8	0.1	Cat 0.1 Column alone
16.1	24.7	443.4	28.3	3.5	2.0	0.001	Cat 0.001 Column alone
12.6	13.1	110.4	18.3	4.5	5.8	0.1	Grass 0.1 Column alone
70.2	31.5	299.6	48.5	6.2	10.2	0.001	Grass 0.001 Column alone

Experiment 29

Atopic (Grass, HDM)

Experiment: HDM allergen with Hanks (HBSS) as diluent and + anti IL 10

Repeated with cat and grass (anti IL10 not used with grass)

T cells depleted using MACS column

Conducted on day 6

Large variance in controls (tubes 1 and 2) - additional controls added (tubes 28 and 29)

IFN-g	TNF-a	IL-10	IL-5	IL-4	IL-2		
pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml		
15.2	25.7	39.3	52.1	3.3	3.5		Control
9.0	13.1	4.1	40.6	2.0	5.9		Control
	25.9	230.8	35.8	1.5	2.7	0.1	Pos antigen (HDM) 0.1
14.5	58.0	4.2	81.1			0.1	Pos antigen (HDM) +anti IL10 0.1
	23.6	2.2	27.1	2.2		0.1	Neg antigen (cat) 0.1
12.2	23.2		41.9		3.7	0.1	Neg antigen (cat) +anti IL10 0.1
10.3	26.3	7.5	37.4		3.9	0.1	Pos antigen (Grass) 0.1
	16.0	45.3	43.3		4.9	0.001	Pos antigen (HDM) 0.001
10.3	28.9	3.5	71.2	2.6	4.8	0.001	Pos antigen (HDM) +anti IL10 0.001
7.1	14.2	3.1	51.8		4.2	0.001	Neg antigen (cat) 0.001
4.2	22.4	2.6	69.4			0.001	Neg antigen (cat) +anti IL10 0.001
	17.1	1.6	46.5		3.1	0.001	Pos antigen (Grass) 0.001
	5.9	5.3	8.7		2.9	0.001	Pos antigen (Grass) + Dex 0.001
5.0	4.8	6.4	2.5				Control T dep
	8.6	7.7	2.9		4.1		Control T dep
	21.1	147.2	3.1		3.1	0.1	HDM 0.1 T dep
	5.5	10.5	2.4			0.1	Cat 0.1 T dep
	45.0	80.3	3.6		3.3	0.1	Grass 0.1 T dep
2.1	7.3	63.6	3.1		6.4	0.001	HDM 0.001 T dep
	6.5	15.8	2.7		2.4	0.001	Cat 0.001 T dep
3.3	31.3	54.1	3.9			0.001	Grass 0.001 T dep

6.3	15.6	25.3	42.8		2.7		Control	
3.3	10.6	19.8	47.5		5.2		Control	
4.7	25.9	214.5	45.6	1.7		0.1	Pos antigen (HDM)	0.1
1.3	21.1	185.2	50.8	1.8	2.3	0.1	Pos antigen (HDM)	0.1
	21.7	159.9	46.7		2.3	0.001	Pos antigen (HDM)	0.001
4.5	25.3	228.7	49.3		2.7	0.001	Pos antigen (HDM)	0.001

Experiment 30

Non - Atopic

Experiment: HDM allergen with Hanks (HBSS) as diluent and + anti IL 10

Repeated with cat and grass

T cells depleted using MACS column, with column control

Conducted on day 6

IFN-g	TNF-a	IL-10	IL-5	IL-4	IL-2		
pg/ml	pg/ml	pg/ml	pg/ml	pg/ml	pg/ml		
	8.0	8.1	140.5		2.3		Control
6.8	7.3		161.8		2.3		Control
	10.4	7.5	181.5			0.1	Neg antigen (HDM) 0.1
5.4	11.4	4.2	285.1		6.0	0.1	Neg antigen (HDM) +anti IL10 0.1
	4.6		171.3	2.0		0.1	Neg antigen (cat) 0.1
4.2	8.3	1.5	415.3	3.2		0.1	Neg antigen (cat) +anti IL10 0.1
3.9	6.8		145.8	2.3	2.0	0.1	Neg antigen (Grass) 0.1
6.3	16.2	1.7	257.1			0.1	Neg antigen (Grass) +anti IL10 0.1
6.6	9.3		150.0		4.1	0.001	Neg antigen (HDM) 0.001
	11.4	3.8	318.3	2.5		0.001	Neg antigen (HDM) +anti IL10 0.001
	5.7	1.8	155.0		3.5	0.001	Neg antigen (cat) 0.001
1.3	6.4	2.8	376.1	1.4		0.001	Neg antigen (cat) +anti IL10 0.001
11.6	6.9		174.6	3.5	6.2	0.001	Neg antigen (Grass) 0.001
7.2	12.1	1.9	246.8		5.1	0.001	Neg antigen (Grass) +anti IL10 0.001
3.9	3.9	3.7	3.9	3.2	2.5		Control T dep
8.4	4.6		3.4		5.0		Control T dep
2.9	5.6	3.1	3.5		4.6	0.1	HDM 0.1 T dep
	4.3	3.9	3.7	2.3	4.1	0.1	HDM 0.1 T dep
9.3	3.4	2.5	3.4	2.3	4.9	0.1	Cat 0.1 T dep
13.8	3.6	2.2	3.5		3.3	0.1	Cat 0.1 T dep
	2.4		3.2	1.6		0.1	Grass 0.1 T dep
4.8	5.7		4.1	3.4	2.0	0.1	Grass 0.1 T dep
	3.9	2.0	3.2		3.0	0.001	HDM 0.001 T dep
7.5	5.0	1.7	3.8	2.5	6.0	0.001	HDM 0.001 T dep

	2.7	2.3	4.0	3.4	4.0	0.001	Cat 0.001 T dep
9.2	5.7	2.1	4.0	3.4	3.5	0.001	Cat 0.001 T dep
4.7	4.7	1.9	3.5	2.8	5.1	0.001	Grass 0.001 T dep
9.5	5.7	2.4	4.2	2.6		0.001	Grass 0.001 T dep
109.2	21.0	422.9	775.0	2.0	4.6		Control T Column alone
304.2	25.3	422.9	737.4		7.6		Control T Column alone
82.8	14.6	455.3	804.7			0.1	HDM 0.1 T Column alone
44.2	11.7	447.0	246.8		2.7	0.1	HDM 0.1 T Column alone
10.3	6.9	81.9	126.9		3.5	0.1	Cat 0.1 T Column alone
15.2	8.2	46.2	67.1		2.6	0.1	Cat 0.1 T Column alone
204.3	18.3	282.2	280.7	2.2	7.1	0.1	Grass 0.1 T Column alone
162.9	21.8	320.9	330.4		5.6	0.1	Grass 0.1 T Column alone
129.9	14.8	368.3	470.0	2.2	1.7	0.001	HDM 0.001 T Column alone
209.1	11.1	323.8	660.5		3.4	0.001	HDM 0.001 T Column alone
94.8	14.5	219.4	310.0	1.7	2.5	0.001	Cat 0.001 T Column alone
92.7	13.6	204.9	562.2	2.3		0.001	Cat 0.001 T Column alone
141.7	20.7	303.7	313.3			0.001	Grass 0.001 T Column alone
84.7	17.7	340.6	621.9	1.4	3.5	0.001	Grass 0.001 T Column alone
147.5	25.0	667.0	424.7	2.2	2.9		Control
82.8	23.9	579.6	158.7	4.1	11.4		Control
105.2	31.6	630.5	291.1	1.4	4.6	0.1	HDM 0.1
39.9	30.4	598.9	332.2	2.4	3.0	0.1	HDM 0.1
55.3	22.5	459.5	356.2		5.7	0.001	HDM 0.001
105.8	31.6	504.1	406.2		3.3	0.001	HDM 0.001