Interleukin-22
in human allergy and asthma

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Pascal Venn

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Supervisors

Prof. Carsten Schmidt-Weber

Dr. Paul Lavender

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Department of Allergy and Clinical Immunology
MRC and Asthma UK centre in Allergic Mechanisms of Asthma
National Heart and Lung Institute, Faculty of Medicine
Imperial College London

Abstract

Interleukin 22 (IL-22) is a newly-described T helper cytokine which has been implicated in tissue regenerative processes in the gut and skin. Allergic asthma results from exposure to otherwise harmless environmental inhaled allergens that trigger Th_2 T lymphocyte-driven IgE-associated immune responses in atopic individuals that contribute to airway inflammation and reversible airflow obstruction. In the long-term, this aberrant immune response is believed to increase in complexity and contribute to the permanent changes in the airways, including tissue remodelling and associated irreversible airway obstruction. In view of the known effects of IL-22 in wound healing, the overall aim of this research was to establish whether IL-22 is present in allergy and allergic asthma and to understand the influence of IL-22 on structural cells of the airway, with particular emphasis on tissue remodelling. We, therefore, explored the presence of IL-22 and its receptors during allergen-induced late responses in the bronchi and skin and measured IL-22 production and its regulation in peripheral blood mononuclear cells (PBMCs), T cell lines and clones derived from asthmatic bronchial mucosa. IL-22 was increased in bronchoalveolar lavage after allergen inhalation in asthmatics but not in control subjects. While we were unable to reliably immunostain IL-22, its receptors were present within the cutaneous connective tissue and co-localised with HSP-47, a marker of early fibroblasts. In PBMCs, IL-22 increased after allergen stimulation whereas there was no difference between atopics and control subjects. In T cell lines derived from asthmatic bronchial mucosa, IL-22 was mainly found in $CD4^+$ T helper cells of Th_1 and Th_{22} origin. IL-22 receptors were expressed by cultured bronchial epithelial cells, smooth muscle cells and pulmonary fibroblasts and were increased in expression in the presence of interferon-γ (IFN-γ). IL-22 was able to accelerate wound closure of bronchial epithelial cells independent of effects on cellular proliferation. Thus, IL-22 appears to be a Th_1-related cytokine that may have a dual role in airway inflammation and repair.

Declaration of Originality

The following thesis is based on my own work and else has been appropriately referenced.

London,12th October 2012

Pascal Venn

Contributions:

The bronchial segmental and intradermal allergen provocations were carried out by the medical staff of the Upper Respiratory Medicine group at the Royal Brompton Hospital under the supervision of Prof. Stephen R Durham. The clinical data as well as eosinophil counts were kindly provided by Dr. Stephen Till (bronchial segmental challenge) and Dr. Graham K Banfield (intradermal challenge).

All the following experiments were designed, validated, carried out, and analysed by myself. These included the development of the IL-22RA2 ELISA, the measurements of cytokine expression within the bronchoalveolar lavage, the immunofluorescent staining of cutaneous biopsies and their analysis, the isolations of T cell populations from peripheral blood, the creation of T cell lines and clones from bronchial tissue, all cell culture work including the allergen stimulation of PBMCs and subsequent measurement of [methyl^{3}H]-thymidine incorporation, the RT-PCR analysis and design of missing primer pairs, the flow cytometric staining and analysis, the validation and analysis of protein expression by western blotting, the development of functional assays such as the wound healing assay, and the establishment of cell cycle analysis of adherent structural cells.

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To

my wonderful Reetu

& my family

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Abbreviations

Abbreviation	Name
%	percentage
AHR	Aryl hydrocarbon receptor
AIM-V	Adoptive immunotherapy media V
ALT	alanine transaminase
APC	Antigen presenting cell
ARDS	Acute respiratory distress syndrome
aSMA	alpha smooth muscle actin
AST	Aminotransferase
BAL	bronchoalveolar lavage
BCA	Bicinchoninic acid
BCL-2	B-cell lymphoma-2
BMP	Bone morphogenic protein
BSA	Bovine serum albumin
CCR	Chemokine C-C motif receptor
CD	Cluster of differentiation
cDNA	copy Deoxyribonucleic acid
CPM	counts per minute
CRTAM	Class-I MHC-restricted T-cell-associated molecule
CTLA4	Cytotoxic T-Lymphocyte Antigen 4
DAPI	4',6-diamidino-2-phenylindole
DMSO	Dimethyl sulfoxide
dNTP	Deoxyribonucleotide
EAE	experimental autoimmune encephalomyelitis
ECL	Enhanced Chemoluminescence
EDTA	Ethylenediaminetetraacetic acid
EGF	Endothelial growth factor
ELISA	Enzyme-linked immunosorbent assay
EMTU	epithelial-mesenchymal-tropic unit
EPO	Erythropoietin
ERK	Extracellular signal-regulated kinases
FACS	Flow assisted cell sorting
Fc R	Immunoglobulin Fc (Fragment, crystallizable) region receptor
FCS	Fetal calv serum
FEV_1	one second forced expiratory volume,
FGFR1	Fibroblast growth factor receptor 1
FICZ	tryptophan photoproduct 6-formylindolo[3,2-b]carbazole
FOXP3	Forkhead box P3
$G_{0/1}$	Cell cycle Gap phase 0/1
$G_{2/M}$	Cell cycle Gap phase 2/Mitosis phase
GATA3	GATA binding protein 3
GMCSF	Granulocyte macrophage colony-stimulating factor
GP	general practitioner
HCL	Hydrogen chloride
HIV	Human immunodeficiency virus
HMBPP	phosphoantigen (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate
HRP	Horseradish peroxidase
HSP-47	Heat shock protein-47
ICAM	Intercellular adhesion molecule
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IRF4	Interferon regulating factor 4’
IU	International units
JAK	Janus kinase

JNK	c-Jun N-terminal kinases
kDA	kilo Dalton
LFA1	Lymphocyte function-associated antigen 1
LPS	Lipopolysaccharide
LS	large sized
LTi	Lymphoid tissue inducer
M	Molar
MACS	Magnetic bead associated cell sorting
MBP	major basic protein
MCT	Mast cell tryptase
MDM1	Mdm4, transformed 3T3 cell double minute 1, p53 binding protein
MES	2-(N -morpholino)ethanesulfonic acid
MHC	Major histocompatibility complex
MMP	Matrix metalloproteinase
MOG	myelin oligodendrocyte antigen
mRNA	messenger Ribonucleic acid
MW	Molecular weight
NE	Neutrophil elastase
NK cell	Natural killer cell
NOS2	Nitric oxide synthase, inducible
OCT	Optimal cutting temperature
OD	optical density
OVA	Ovalbumin
PBMCs	peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PC20	Provocating Concentration 20
PDGFR	Platelet derived growth factor receptor
PGE2	Prostaglandin E2
PHA	Phytohaemagglutinin
Phl p	Phleum pratense
PI	propidium iodide
PMA	phorbol 12-myristate 13-acetate
PPD	Tuberculin purified protein derivative
RAG	Recombination activating gene
RAST	Radioallergosorbent test
RORC2	Rar related orphan nuclear receptor C 2
RPM	rotations per minute
RPMI	Roswell Park Memorial Institute medium
RT-PCR	Real Time polymerase chain reaction
SAA	serum amyloid A1
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SLE	systemic lupus erythemotsus
SNP	Single-nucleotide polymorphism
SOCS	suppressor of cytokine signaling proteins
S-phase	Cell cycle synthesis phase
STAT	Signal transducer and activator of transcription
TB	Tuberculosis
TBET	T-box expressed in T cells
TCR	T cell receptor
TGF	transforming growth factor
Th	T helper
TIE1	Tyrosine kinase with immunoglobulin-like and EGF-like domains 1
TLR	Toll like receptor
TMB	5,5'-Tetramethylbenzidine
TNF	tumer necrosis factor
TRAPS	TNF-receptor-associated periodic syndrome
VEGF	Vascular endothelial growth factor
VCAM-1	vascular cell adhesion molecule-1
v/v	Volume/Volume

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Introduction

Allergy and Asthma

Allergy

Background
Definition

Allergy is an adverse immune reaction to a substance in the environment (allergen) which is normally harmless. In most allergies, this process is characterised by the production of allergen-specific IgE and the development of a Th_2 cell memory. In sensitised individuals, allergen contact provokes a biphasic inflammatory response that can be divided into early and late phases. The early phase response results from IgE-dependent mast cell activation with release of mediators that, depending on the site of exposure, cause discomfort to the skin (itch, wheals), nose (sneeze and watery discharge) and lung (wheeze and shortness of breath). The late response is T-cell mediated and characterised by eosinophilic infiltration. This response is accompanied by further symptoms including swelling, mucus overproduction, as well as airway hyperreactivity.

Epidemiology

In the United Kingdom, the overall prevalence of allergic rhinitis has been estimated at 23 percent (%) (Bauchau and Durham 2004). It is estimated that, in England, each year about 3 million people consult their general practitioner (GP) with conditions related to allergy. The prevalence of multiple allergic diseases was observed to increase by 48.9% between the years 2001 and 2005 (House of Lords - Science and Technology - Sixth Report 2007).

Mechanisms of allergy

There are three main processes which have been identified to be altered in allergic individuals. These include weakened epithelial barriers (Wang *at el* 2008b), a predisposition to produce Immunoglobulin class E (IgE) and an inability to regulate the aberrant immune response (Robinson 2009). These three processes are controlled not by one but multiple genetic and environmental factors. Alterations in the immune system are manifested systemically throughout the body yet allergic disorders are often organ specific (e.g. skin, gut, and airways) based on the route of allergen exposure and organ or tissue specific alterations.

Genetic versus Environmental influences

Family studies have shown that allergic disorders follow a hereditary pattern (Skadhauge *et al* 1999, Hanson *et al* 1991, Lawrence *et al* 1994). It was once hypothesized that the basis of altered IgE responses was inherited as an autosomal dominant trait (Cookson and Hopkin 1988). Subsequent studies revealed that the clinical manifestations of allergy are not controlled by a single gene but, rather, multiple smaller genetic variations that are often influenced or directly controlled by environmental factors (Vercelli *et al* 2005).

Allergy is, thus, believed to have a genetic component but dependent on multiple environmental factors. While environmental insults (e.g. malnutrition, stress, chronic illness, pollution, exercise and viral infections) are known to influence the threshold for disease manifestation temporarily as well as permanently, events in early life are believed to be particularly damaging and influence the progression from a predisposed to allergic state.

In allergy, the deficient regulation of the Th_2 response is thought to have occurred due to altered environmental exposures in early life when the adaptive immune system and immunological memory is in development. The fact that immunologic tolerance may be re-established in allergic individuals through immunological interventions such as allergen immunotherapy implies that allergic disease progression is under the control of environmental factors.

The hygiene hypothesis suggests a link between the development of allergy (and loss of immune tolerance to common environmental allergens) with exposure to an altered microbial flora during early life. The hypothesis was based on the observation of an inverse relationship between family size and the risk of developing atopic disorders (Strachan 1989), younger siblings being 'protected', possibly by increased exposure to infections. Support came from the higher prevalence of allergic diseases that were associated with a more 'westernised' lifestyle. This was originally observed as a much lower prevalence of atopic sensitisation and hay fever in underdeveloped Leipzig in East Germany compared to Munich (von Mutius *et al* 1992) whereas within 5 years of the re-unification of East and West Germany, the prevalence had tended to equalise (Weiland *et al* 1999). Our modern way of living (urbanisation, sanitation, dietary changes and medication) has been associated with a marked reduction in our natural microbial environment. It has been suggested that the loss of toll-like receptor stimulation during early life may alter the development of our acquired immune system from a Th_1-biased microbial protective state to a more polarised Th_2-type response (Strachan 2000), whereas, more recently, it has been suggested that the observed increase in both allergic disorders and auto-immune diseases may be explained by a

decrease in T regulatory cell function, resulting in unrestrained Th_1- (and Th_{17}-) and Th_2-biased immune responses (Kamradt *et al* 2005). These alterations might provide the basis for progression from a predisposed to an allergic state (Eder *et al* 2006).

While this hypothesis has received much support (Ege *et al* 2006, Waser *et al* 2007), it also has been challenged with suggestions that allergic disease progression cannot be explained by reduced microbial exposure alone (Platts-Mills *et al* 2005, Bresciani *et al* 2005).

Pathogenesis

Sensitisation

Before an allergic response can develop, the immune system must first encounter the allergen and develop specific immunological memory against the allergen. In an allergic individual, dendritic cells within the mucosal epithelium will capture the allergen, recognise it as harmful and migrate to the draining lymph node (Takano *et al* 2005). The, now, activated dendritic cells present the processed allergen through an Major histocompatibility complex (MHC) class II-mediated mechanism to allergen-specific T helper cells. This priming will predominantly induce Th_2 cells capable of releasing IL-4, IL-5 and IL-13 (Romagnani *et al* 2002). Allergen-specific Th_2 cells in turn licence allergen specific B cells to class switch their B cell receptor in favour of allergen specific IgE production (Kay 2001, Gould *et al* 2003). This allergen-specific IgE in the circulation binds to high affinity Fcε receptors on various innate cells, including mast cells and basophils. These FcεR1-expressing cells are now poised to recognise the allergen upon secondary encounter.

Acute phase response

The secondary exposure will lead directly to recognition of allergen by resident mast cells and basophils within the exposed tissue. Upon cross-linking of the allergen with the surface bound IgE, these cells will degranulate and release several mediators including vasoactive amines (e.g. histamine), cytokines, chemokines, reactive oxygen species, and lipid mediators such as leukotriene C4, D4, and E4 as well as prostaglandin D2 (Kraft *et al* 2003). This immediate release of inflammatory mediators is referred to as type-1 hypersensitivity (Gell and Coombs 1963) and is responsible for the immediate clinical symptoms such as itching (atopic dermatitis), nasal congestion (allergic rhinitis), and airway constriction (allergic asthma) (Bradding *et al* 1992, Pease 2006) within minutes of allergen exposure.

Late phase response

Following the early response, a proportion of individuals go on to develop a delayed-in-time response (the allergen-induced late response) peaking between 6 and 12 hours. The late response is characterised by large cellular infiltration caused by the release of

chemoattractants during the early phase including eosinophils, and $CD4^+$ T lymphocytes, as well as other immune cells at the site of allergen exposure (Kay 2001). The influx of T cells, particularly Th_2 cells produce Th_2 cytokines. These include IL-4 resulting in Th_2 T cell differentiation and local IgE-switching (Takhar *et al* 2005), IL-13 IgE-switching, mucus hypersecretion (Lachowicz-Scroggins *et al* 2010) and, along with IL-4, there is an increase in the expression of vascular cell adhesion molecule-1 (VCAM-1). Furthermore, IL-5 release results in tissue eosinophilia during the late-phase immune response resulting in further clinical symptoms through tissue swelling and mucus hypersecretion. T cells, in particular, hold a critical role in orchestrating the late phase response which can be observed even in the absence of mast cell activation and eosinophil infiltration (Ali *et al* 2007).

Treatment

For allergic rhinitis, when avoidance of the provoking allergen is not possible, the use of anti-histamines and topical corticosteroids in combination with symptoms-relieving medication is prescribed. Similarly, asthma is treated with corticosteroids and beta-agonists for symptomatic relief. However, these treatments are for symptomatic use and have no effect on the long-term course of the disease. The only long-term disease-modifying treatment option available, so far, is allergen immunotherapy (Ewbank *et al* 2003, Holgate and Polosa 2008). Immunotherapy aims to reduce allergic symptoms by inducing immunologic tolerance and has been shown to be effective leading to long-term benefits (Robinson *et al* 2004, Shamji and Durham 2011) however it is not used in allergic asthma due to safety concerns.

Asthma

Background

Definition

Bronchial asthma is a respiratory disorder characterised by reversible airflow obstruction either spontaneously or as a result of treatment. In asthma, the airways are hypersensitive to certain endogenous and exogenous stimuli that trigger spontaneous muscle contraction with associated airway constriction. The airway narrowing, in asthma, occurs in the context of airway inflammation that is typically characterised by bronchial mucosal eosinophilia, disruption of the integrity of the epithelium, mucus hypersecretion, thickening of the sub-basement membrane zone and smooth muscle hypertrophy that increases bronchial wall thickness. The progressive loss of airway function, particularly in chronic asthma, is often associated with aberrant changes in the lung described under the term airway remodelling.

Epidemiology

In England, 5.7 million people suffer from asthma (House of Lords - Science and Technology - Sixth Report 2007). As for other disorders associated with allergy, a relatively high prevalence of asthma is found in 'westernised' countries (about 15% to 20%) while the prevalence in developing countries remains much lower (about 2% to 4%) (Zock *et al* 2006). Very recently, this trend has changed and the prevalence of asthma has shown signs of levelling off in western countries (Moorman *et al* 2007) while it continues to rise in developing countries (Pearce *et al* 2007).

Endophenotypes of asthma

The current definition of asthma encompasses a diverse group of conditions which do not always share the same underlying disease mechanism or progression. Characterising asthma by phenotype (e.g. Eosinophilic vs. Neutrophilic, Early vs. Late-onset, Moderate vs Severe) helped to understand this diversity allowing a more tailored treatment. Yet the phenotypic classification alone remains insufficient in fully understanding the molecular mechanisms and differences in treatment outcomes. The advent of large scale genetic and genomic studies has helped to shed more light on common molecular patterns between different patient groups. This molecular characterisation of asthma patients has prompted researchers to redefine asthma into endophenotypes (see table 1; Loetvall Akdis 2011, Anderson 2008, Wenzel 2012, Poon Hamid 2012). This classification combines the emerging molecular insight together with the phenotype and observed treatment response. While endophenotype classifications promise a more targeted intervention (Kazani and Israel 2012) their definition remain ongoing (see table 1).

Table 1: Proposed Endophenotypes of Asthma

Asthma	Endophenotypes	Reference
General	Aspirin-sensitive Allergic bronchopulmonary mycosis Adult allergic Asthma-predictive indices-positive preschool weezer Severe late-onset hypereosinophilic Cross-country skiers asthma	Lotvall et al 2011
Severe Asthma	Early-onset allergic Persistent eosinophilia Allergic bronchopulmonary mycosis Obese-female Neutrophilic severe asthma	Wenzel et al 2012

Development of asthma

Asthma is a complex disorder controlled by multiple minor and major factors which are both inherited as well as acquired in early life. Alterations of the epithelial cell function, its barrier integrity, innate defence and repair response are thought to be some of the main underlying mechanisms of asthma. There is a strong relationship between allergy and asthma as the allergic airway inflammation can act as the asthmatic trigger for bronchoconstriction. Both atopic disorders and asthma are often characterised by a dysfunctional epithelium (Holgate *et al* 2009). However, allergy or atopy is not a prerequisite to develop asthma and, thus, allergic asthma forms a separate sub phenotype of asthma.

Genetic and environmental determinants of asthma

Family studies have confirmed that asthma follows a hereditary pattern (von Mutius *et al* 1996, Laitinen *et al* 1998). The contribution of genetic associations has been the subject of numerous genome wide association studies and, in particular, early-onset asthma shows a strong influence of genetic inheritance (Moffatt *et al* 2007 & 2010). It is now believed that the genetic alterations underlying asthma are predominantly related to genes altering epithelial, mesenchymal and smooth muscle cell function (Cookson 2004, Vendelin *et al* 2005, Holgate and Polosa 2008). However, environmental factors are known to play the major role in disease expression in asthma. For example, studies of migrating families have shown that the duration of residence in a new environment can alter asthma prevalence in young and newborn children (Cohen *et al* 2007, Wang *et al* 2008a, Wong and Chow 2008). The development of asthma is influenced, particularly, by environmental factors in early life. While changes to the still developing immune system can contribute to the development of allergy, the development of asthma may additionally depend on factors that influence normal lung development in prenatal (Sporik *et al* 1990, Dietert and Zelikoff 2008, Patel and Miller 2009) and early life (Sigurs *et al* 2005, Carroll *et al* 2009). The risk factors are very diverse

and include air pollution (Tzivian 2011), viral (Johnston *et al* 2007) and bacterial infections (Cosentini *et al* 2008), nutrition (Noal *et al* 2011), medication (Nuttall *et al* 2000), stress (Sandberg *et al* 2000) amongst other factors.

Pathogenesis

An asthma attack results from a loss of lung function (fall in peak expiratory flow rate and the one second forced expiratory volume, FEV_1) that causes mild to severe difficulties in breathing. It is associated with the rapid contraction of airway smooth muscle leading to narrowing of the bronchi. 'Brittle' asthma (Paganin *et al* 1996) represents an asthma phenotype characterised by very rapid onset of airway narrowing which may be largely explained by smooth muscle constriction. With the exception of pauci granulocytic asthma, cellular infiltration and tissue inflammation is also observed during acute asthma. Swelling and inflammation further contributes to the difficulty in breathing.

A diverse range of stimuli including allergen, cold air, exercise and irritants may represent triggers that may provoke smooth muscle constriction and bronchial wall thickening with airway narrowing via inflammatory or non-inflammatory events. The threshold for bronchoconstriction is influenced by the level of nonspecific bronchial hyperreactivity which, in turn, can be influenced by numerous factors such as chronic allergen exposure, viral infections, diet, stress, and medication. Nonspecific bronchial hyperreactivity can be assessed by inhalation challenge with histamine or methacholine and expressed as the provocation concentration of inhaled agent that provokes a 20% fall in FEV_1 (e.g. histamine PC20 (Juniper *et al* 1978).

In allergic asthma, the encounter of allergen will lead to clinical symptoms such as airway narrowing and airway inflammation. The allergic response is often biphasic, leading to an early and late asthmatic reaction.

The early phase occurs within minutes, leading to acute bronchoconstriction. It is initiated by IgE-mediated allergen recognition on the surface of mast cells and basophils leading to their activation, rapid degranulation and release of mediators of hypersensitivity (eg. histamine, prostaglandins, leukotrienes). Mast cells, which are widely distributed throughout the tissue including the smooth muscle tissue, release potent smooth muscle contractile agents, initiating bronchoconstriction (Holgate 1996). In addition to mast cell activation T cell recognition will lead to a burst of Th_2-type cytokines (e.g. IL-4, IL-5, IL-13) influencing the early as well the ensuing late phase response (Wenzel *et al* 2007).

The late asthmatic response is experienced by half of allergic asthmatics and causes a second episode of airway narrowing but with marked differences in symptoms and features

to the early asthmatic response (Robertson *et al* 1974, O'Byrne *et al* 1987). This delayed reaction to the allergen, which is displayed at about 3-8 hours after the initial allergen encounter, is characterised by inflammation, swelling, mucus over-production and significantly airway hyper reactivity. This airway hyper reactivity can resolve within 24 hours but occasionally can last for several days or even weeks following a single allergen exposure (Cartier *et al* 1982, O'Bryne *et al* 1987). The inflammation which is displayed during the late phase response is caused by recruitment of eosinophils (De Monchy *et al* 1985, Gauvreau *et al* 1999) as well as T cells (predominantly Th_2 phenotype) into the airways (Robinson *et al* 1993).

The symptoms of the late phase response are largely controlled by the preventive use of corticosteroids indicating a clear functional role of the immune system (Cockroft *et al* 1987, Kidney *et al* 1997). The blockade of the hallmark eosinophilic infiltration reduces asthma exacerbations (Green *et al* 2002), signs of airway injury and remodelling (airway-wall thickness and total wall area), but not airway hypersensitivity (Leckie 2000, Haldar 2009). The airway hypersensitivity depends on the early phase IgE-mediated immune cell activation (Cartier *et al* 1982, Kirby *et al* 1986, Fahy *et al* 1997) and subsequent mediator release (Wenzel *et al* 2007). However its later mechanisms and occurrence during the late phase remain far less understood. One avenue which has been revisited recently is the potential activation of sensory nerves during the early phase. This early activation may, through delayed reflex responses, account for airway hypersensitivity during the late phase response (Pavord and Tattersfield 1995, Tilley *et al* 2003, Raemdonck *et al* 2012).

Airway remodelling

The progression of the asthma is associated with changes in the underlying cellular composition of and structure of the bronchial epithelium and submucosa. These aberrant changes (Table 2) are often described under the term airway remodelling and may cause a further reduction in the overall lung function with more severe exacerbations (Jain *et al* 2005) and the development of an irreversible component.

Table 2. Structural alterations and airway remodelling

Cell type	Observation	Effect
Goblet cells	Number of goblet cells increase Mucin 5AC dominates and are largely responsible (Morcillo and Cortijo 2006)	Reduction of airflow through difficulty in expectoration of sputum
Smooth muscle	Hypertrophy and hyperplasia.	Narrowing of the airways due to bronchial wall thickening with luminal narrowing and increased bronchoconstriction during an asthmatic attack
Fibroblasts	Myofibrobalst increase (Choe *et al* 2006, Wicks *et al* 2006),	Increased scarring
Vessels	Angiogenesis leading to an increase in microvessels (Zanini *et al* 2010)	Oedema of the bronchial wall with narrowing of the airways, increased immune and inflammatory cell recruitment.
Epithelial cells	Epithelial damage, decrease in tight junctions and increase in permeability (Montefort *et al* 1992).	Decrease in barrier function and increased risk of inflammation and exposure to asthmatic triggers
Basal lamina	Extracellular matrix proteins and proteoglycan deposition (Knight and Holgate 2003)	Increased thickening

The presence of persistent changes within the airway wall is believed to be controlled by inherent and acquired defects connected to aberrant tissue repair responses.

Non-Immune contribution

The structural cells within the asthmatic lung display altered physiological functions associated with oxidant handling (Bayram *et al* 2002, Bucchieri *et al* 2002), inflammation (Mullings *et al* 2001, Sampath *et al* 1999) and repair (Amishima *et al* 1998, Payne *et al* 2004). This altered functionality is believed to influence the normal resolution of airway inflammation, alter the normal repair processes and cause permanent structural changes within the lung.

Signs of this aberrant airway remodelling were detectable in children at risk of developing asthma even when inflammation was moderated by corticosteroid use and long term use of corticosteroids did not significantly prevent the progression of asthma (Pauwels *et al* 2003, Bisgaard *et al* 2006, Guilbert *et al* 2006, Murray *et al* 2006).

These early paediatric studies especially support the notion that airway remodelling is not necessarily caused by inflammation but may occur in parallel or even precede airway inflammation. The aberrant healing and repair processes observed within the asthmatic lung are believed to be similar to events occurring during early lung development. One hypothesis is that airway remodelling could be caused by reactivation of embryological pathways, in particular the epithelial-mesenchymal-tropic unit (EMTU) causing aberrant and irreversible structural alterations (Holgate and Polosa 2006). While the hypothesis of a re-activated EMTU has gained some recognition, it remains difficult to confirm.

Airway remodelling, either controlled by re-activated embryological pathways or otherwise caused by functional damage in structural cells, is likely influenced by inheritance and environmental exposure (Lloyd and Robinson 2007). While inherited genetic alterations in asthmatic epithelial cell function are likely providing a predisposition for airway remodelling, one theory is that particularly severe and repeated respiratory infections in early life are a risk factor. These viral infections may overwhelm the inexperienced immune system causing significant damage to the developing lung and provide the necessary initial insult for airway remodelling to occur and progress (Openshaw and Hewitt 2000).

Immune contribution

Inflammation remains the hallmark of asthma and allergic asthma is associated with Th_2 type inflammation. The release of tissue damaging mediators such as superoxide and nitric oxide (e.g. by mast cells, eosinophils and basophils) cause cellular damage and the release of potent cytokines (e.g. by T cells) alter the functional state of structural cells (Vignola *et al* 2000). In the normal lung, these changes would be almost always resolved without leaving significant long term effects. In the asthmatic lung, however, aberrant tissue repair processes are observed. The occurrence of repetitive or chronic inflammation is thought to trigger these processes and exacerbate airway remodelling (Holgate 2006). The moderation of inflammation by the help of corticosteroid use caused a reduction in epithelial damage (Lundgren *et al* 1988, Gavett *et al* 1994), goblet cell hyperplasia (Chanez *et al* 2004), micro vessel formation (Feltis *et al* 2007), subepithelial fibrosis and basement membrane thickness (Trigg *et al* 1994, Olivieri *et al* 1997, Laitinen *et al* 1997).

Treatment
Current interventions

For asthma two distinct types of mediation are being prescribed (see Table 3). They provide either immediate relief (e.g. reliever medication) or reduce or prevent the potential symptoms during the encounter of a future asthmatic trigger (e.g. preventer mediation).

Reliever medication

Reliever medication reverses the airflow obstruction dilating the bronchi. They are thus often referred to as bronchodilators and encompass short-acting β2 adrenergic receptor agonists, anti-cholinergic drugs, as well as dimethylxanthine.

Short-acting β2 adrenergic receptor agonists (also referred to as β2 agonist) are the mainstay medication and reverse smooth muscle constriction by binding and activating the beta-adrenergic receptors on the smooth muscle fibres. Anticholinergic drugs on the other

hand interfere with the signal transmission from the nerve ending to the smooth muscle fibre. This blockade of acetylcholine signalling allows the smooth muscle to relax. As they act slower than β2 agonist, the two types of drugs are often combined. Theophylline (or dimethylxanthine) is another reliever medication which is a non-specific antagonist of the adenosine receptor and inhibitor of phosphoesterase. Both actions will cause the downstream inactivation of myosin light chain kinase and phosphatase leading to smooth muscle relaxation.

Symptom controllers

Long-acting β2 agonist provide muscle relaxation for up to 12 hours and are thus used to control symptoms that may occur later on or during night time. They are often combined with a type of preventer medication providing a more comprehensive symptom control.

Preventer medication

Preventer mediation aims to reduce the severity of airway inflammation and immune-mediated airflow obstruction. They include steroid analogues (e.g. of glucocorticosteroids) as well as non-steroidal drugs (such as mast cell stabilisers and leukotriene modifiers).

Glucocorticosteroid analogues (also refrerred to as glucocorticoids) mimic the effect of the natural occurring hormones within the human body. As regulators of the glucose metabolism they have wide ranging physiological effects. Most importantly, they interfere with immune cell activation (Coutinho and Chapman 2011). The preventive use of their analogues is thus effective in dampening the inflammatory response which would normally occur during an asthma attack and trigger clinical symptoms (Rhen and Cidlowski 2005). To restrict their effects, gluococorticoids are often administered locally using an inhaler. However, if inhalation provides insufficient control, oral administration in either tablet or liquid form may be prescribed. Non-steroidal drugs, often regarded as less effective than glucocorticoids, represent an alternative when the side-effects of the broad-acting steroids (e.g. osteoporosis, cardiovascular and metabolic disease; Vegiopoulos and Herzig 2007) are judged to be too great (e.g. in children; Fanta *et al* 2009). They include mast cell stabilisers as well as leukotriene modifiers. Mast cell stabilisers prevent mast cell degranulation and mediator release but also influence neural reflexes and cytokine transcription (Heinke et al 1995, Yang 2010). Leukotriene modifiers such as Leukotriene receptor antagonists and 5-lipoxygenase synthesis inhibitors limit the activity of the crucial family of inflammatory mediators (incl. Leukotriene D4), limiting vascular permeability, immune cell recruitment, as well as smooth muscle constriction.

Table 3: Current medications used to control asthma

Medication	Example	Mechanism
Reliever Medication	Short-acting β2 adrenergic receptor agonist *e.g. salbutamol, terbutaline*	Bind & activate beta-adrenergic receptors on smooth muscle leading to cAMP production, intracellular Ca^{2+} depletion and smooth muscle relaxation (Taylor 2006)
	Anti-cholinergic drugs *e.g. ipratropium bromide*	Blocks muscarinic acetylcholine receptors & acetylcholine signalling causing smooth muscle relaxation (Baigelman, Chodosh 1977)
	Theophylline (dimethylxanthine)	Non-selective phosphoesterase & adenosine receptor antagonist causing increased cAMP & cGMP activity and smooth muscle relaxation (Daly 1986, Essayan 2001)
Symptom Controller	Long-acting β2 agonist (+preventer medication) *e.g. formoterol,salmeterol (+e.g. glucocorticoids)*	Same as short-acting beta2 agonists. Relieve bronchospasm for up to 12 hrs
Preventer Medication	Steroidal drugs • Inhaled glucocorticosteroid analogues *e.g. beclomethasone dipropionate, budesonide, fluticasone propionate* • Oral glucocorticosteroids analogues *e.g. prednisolon, prednisone*	Agonism of the glucocorticoid receptor. Dampening the activity of key inflammatory regulators such as NF-κB and AP-1 (McKay and Cidlowski 1999) as well as supporting immune regulatory gene transcription (Clark 2007)
	Non-steroidal drug A)mast cell stabilisers *e.g.sodium cromoglycate, nedocromil sodium*	The mechanisms are not fully understood. They reduce Ca2+ channel activity and act as G-coupled receptor agonists. In addition to blocking mast cell degranulation they alter neural reflexes as well as cytokine release (Heinke et al 1995, Yang *et al* 2010)
	B)leukotriene modifiers • Leukotriene receptor antagonists *e.g. montelukast sodium, zafirlukast*	antagonism of the Cysteinyl leukotriene receptor-1 limiting LTD4 but also LTC4, and LTE4 activity (Montuschi and Peters-Golden 2010)
	• 5-lipoxygenase synthesis inhibitors e.g. *zileuton*	inhibit the enzyme arachidonate 5-lipoxygenase reducing the conversion of essential fatty acids into leukotrienes (Pergola and Werz 2010)

New biological treatments for asthma based on immunological insights

The success of corticosteroids has shown that controlling the airway inflammation is an important strategy in the control of asthma. While new and improved broad-acting immune modulators are being developed (e.g. improved corticosteroids, phosphodiesterase inhibitors), more selective and specific immunologic interventions have come into focus (see table 4).

This more selective intervention also requires that the specific underlying molecular mechanisms are well understood. Their potential use and application has increased by the current drive to define better asthma endophenotypes.

In the focus of allergic asthma are especially molecules released during the early phase of inflammation. These include IgE, mast cell mediators as well as Th_2 cytokines. The potency of blocking a single mediator such as a cytokine can be significant as they act not only during the immediate early phase response but also trigger the ensuing late phase response. Anti-IL-5 treatment for example lowered the activation, maturation and recruitment of eosinophils into the airways and significantly reduced asthma exacerbations (Haldar *et al*

2009). Due to the heterogeneity of asthma, not only Th_2 but also Th_1 (e.g. TNFα) as well as Th_{17} (IL-17) cytokines have come into view (Pelaia *et al* 2012).

A different treatment strategy is to use of immunotherapy. Since its conception, the idea of treating the occurrence of allergic inflammation has been very attractive. Yet the risk of anaphylactic reactions, which in allergic asthma could be severe, has hampered its adoption. A systematic review of over 88 clinical trials however has documented significant reductions of asthma symptoms, medication use, and allergen-specific bronchial reactivity after subcutaneous immunotherapy (Abramson *et al* 2003 & 2010). Therefore new developments such as sublingual administration or use of modified allergen (Vrtala *et al* 2001) could potentially lead to a wider adoption in future.

Table 4: New biological treatments for asthma based on immunological insights

Target	Mediators under evaluation	Mechanism
Signalling pathways	Phosphodiesterase inhibitors *e.g. Roflumilast (Gavreau 2011)*	Block phosphodiesterase thus preventing inactivation of cAMP & cGMP within immune cells (Spina 2008)
	p38 MAPK inhibitor s *e.g. SD-282*	Interfere with MAPK signalling cascade dampening cellular responses such as cytokine transcription (Goldstein and Gabriel 2005)
IgE	Anti-IgE *e.g. omalizumab; 8D6 (Shiung 2011)*	Humanised IgG1 antibody binds free but not FcεRI bound IgE. The removal of free IgE causes the gradual loss of receptor bound IgE on Mast cells reducing allergen recognition (Holgate *et al* 2005)
Cytokines	IL-4 • Anti-IL-4 (pascolizumab) • IL-4mutant (pitrakina) • IL-4Ra (altrakinacept)	Blockade of IL-4 function by targeting IL-4 directly, using a decoy receptor or a competitive non-functioning IL-4 mutant
	IL-5 • Anti-IL-5 (mepolizumab) • Anti-IL-5Ra (Benralizumab)	Blockade of IL-5 function by targeting IL-5 directly or using a decoy receptor causing reduction of airway eosinophilia
	IL-13 • Anti-IL-13 (lebrikizumab, Traklokinumab)	Blockade of IL-13 function by targeting IL-13 directly (anti-IL5)
	TNFα • Anti-TNFα (golimumab, Wenzel Barnes 2009) • IgG1/TNFαR2 (etanercept, Erin Leaker 2006)	Blockade of TNFα function by targeting TNFα directly (anti-TNF) or using a decoy receptor
CRTH2	CRTH2 agonist *e.g. OC000459 (Barnes Pavord 2011)*	Interfere with Mast cell mediated activation of Th2 and eosinophils (Gyles 2006)
Immunotherapy	Subcutaneous, sublingual	Reduction of Th2-type inflammation by induction of tolerance e.g. T regulatory cells, blocking antibodies (Shamji and Durham 2011)

T helper cells

CD4 T cells (also called T helper cells) are a group of lymphocytes playing an essential role in the adaptive immune response. They are considered to be bone marrow derived and undergo positive and negative selection within the thymus (Asthon-Rickardt *et al* 1994) to enter the body's circulation as competent naive CD4 T cells. These naive CD4 T cells enter and exit secondary lymphoid tissue (e.g. lymph nodes) where they are brought into contact with professional antigen presenting cells (APC). This interaction may lead to their antigen specific activation (Janeway 1989, Hugues 2010). Depending on the antigen itself, as well the environmental signals which the antigen presenting cell received, the APC will prime the naive T cell through activation of specific transcription factors (e.g. FOXP3, GATA3, T-BET) to acquire specific T helper cell functions (Abbas *et al* 1996, Ho and Glimcher 2002, Rothenberg *et al* 2005). Once activated, the T cell can migrate to the T cell B cell zone to provide help for B cell activation (Parker *et al* 1993) or migrate, guided by chemotactic signals, to the inflamed tissue (Krüger and Mooren 2007). After resolution of inflammation, a small proportion of activated CD4 T cells will remain and form part of the immunological memory (Swain and Bradley 1992).

Current T helper classifications

It was proposed that the different T helper cell profiles, induced during T cell priming, may not only be distinct from each other but also remain stable over time. The first evidence of stable and distinct T helper cell profiles was made when clonal memory T cell lines could be broadly categorised into two stable and distinct IFNγ and IL-4 expressing CD4 T cell populations (Mossmann *et al* 1986, Killar *et al* 1987, Romagnani *et al* 1991). This gave rise to the first Th_1 and Th_2 subset classification and has, since, significantly shaped our understanding of CD4 T biology. The most widely accepted T helper cell subsets today are the Th_1, Th_2, Th_{17} as well as the T regulatory subset (Diagram 1, page 35). In addition, several new classifications have been proposed which include the follicular (Vinuesa *et al* 2005), Th_9 (Dardalhorn *et al* 2008, Veldhoen *et al* 2008), as well as the Th_{22} subset (Duhen *et al* 2009, Eyerich *et al* 2009, Nograles *et al* 2009, Trifani and Spitz 2010).

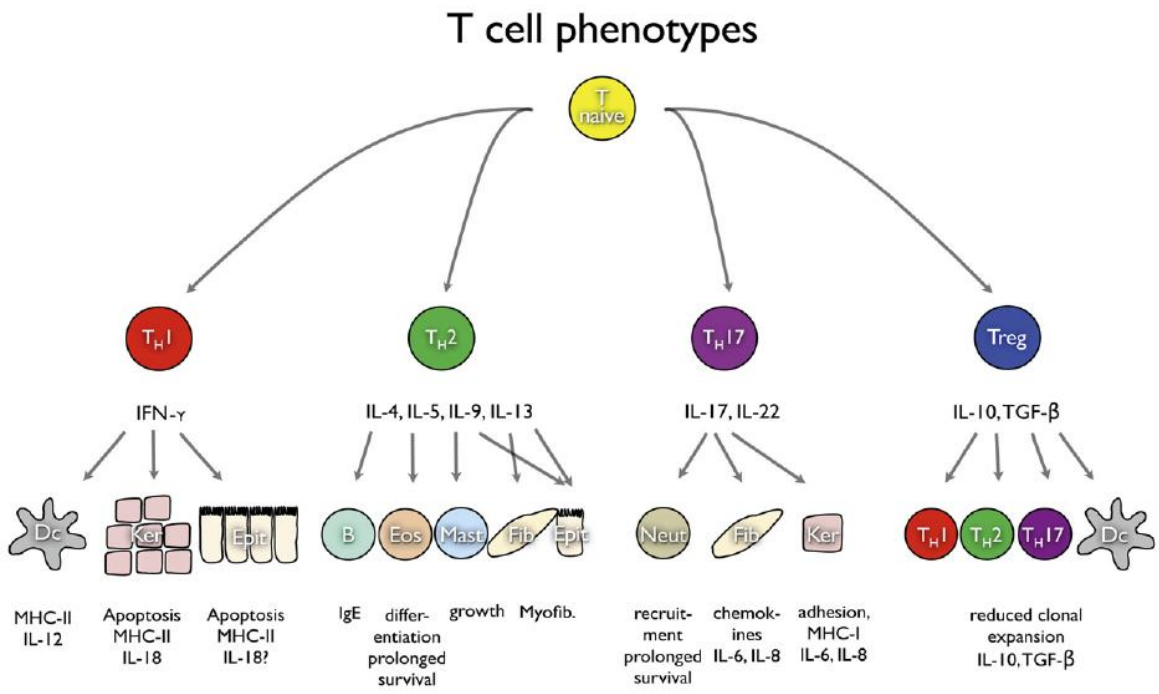


Diagram 1. T cell phenotypes and subset associations (taken from Schmidt-Weber *et al* 2007).

Th_1 and Th_2 subsets

While Th_1 and Th_2 cells were originally characterised by their separate $IFN\gamma^+$ and $IL\text{-}4^+$ production, additional cytokines have since been classified as either Th_1 or Th_2-associated. Examples of these include the Th_1- associated cytokines $TNF\alpha$, lymphotoxin (Cherwinski *et al* 1987, Fiorentino *et al* 1989) as well as Th_2- associated cytokines IL-5, IL-9, IL-13, and IL-31 (Castellani *et al* 2010). With the emergence of more T helper subsets, some of the previously Th_1 (e.g. IL-17, IL-22) and Th_2-associated cytokines (e.g. IL-9, IL-10) have since been reclassified.

In addition to their differential cytokine expression profiles, it was shown that Th_1 and Th_2 cells could be distinguished by different surface receptors. These include chemokine receptors such as CCR5 and CXCR3 (Th_1 associated) and CCR3, CCR4, CCR8, CXCR4 (Th_2 associated) (O'Garra *et al* 1998, Cosmi *et al* 2001).

<u>Th_1 and Th_2 differentiation</u>

IL-12 and $IFN\gamma$ are essential cytokines in the development of Th_1 cells (Hsieh *et al* 1992, Lighvani *et al* 2001). Th_2 cells, on the other hand, only developed in the absence of $IFN\gamma$ and IL-12 when IL-2 and IL-4 are present (le-Gros *et al* 1990, Swain *et al* 1990) inducing further IL-4 release within the differentiating Th_2 cell (Noben-Trauth *et al* 2002).

Transcriptional regulation

As all naive T cells have the capacity to differentiate into any type of T helper cell, the induction of transcription factors, their co-operation and antagonism is important to confer a specific T helper cell identity.

The essential transcription factor for Th_1 development is T-box expressed in T cells (T-bet; Zsabo *et al* 2000) while Th_2 are dependent on GATA binding protein 3(GATA3) expression (Zheng *et al* 1997). The essential transcription factors are often referred to as master regulators which control much wider array of functions related their development as well as specific effector functions. GATA3, for example, was also shown to be essential for Th_2 development and IL-5 and IL-13 cytokine expression (Siegel *et al* 1995, Zhu *et al* 2004). In addition, Signal transducer and activator of transcription (STAT) proteins interact with these transcription factors and are indispensible for T helper fate determination. While the same type of STAT protein can play important roles in several T helper subsets, their strongly increased expression is often subset specific. STAT1 (Lighvani *et al* 2001, Afkarian *et al* 2002) and STAT4 (Kaplan *et al* 1996, Thierfelder *et al* 1996) were shown to be important for Th_1 cells while STAT5 (Zhu *et al* 2003) and STAT6 (Kaplan *et al* 1996, Schimoda *et al* 1996, Takeda *et al* 1996) were essential for Th_2 development. An overview of the Th_1 and Th_2 cell classifications is given in the table below (Table 5).

Table 5. Th_1 and Th_2 subset classification

Subset	Lead Cytokine	Assoc. cytokines	Transcription Factors	Surface Expression	Stat regulation	Polarising cytokines
Th1	IFNγ	TNFα lymphotoxin	T-bet	CCR5 CXCR3 IL-12RB2 IFNγR	STAT1 STAT4	IL-12 IFNγ
Th2	IL-4	IL-5 IL-9* IL-13 IL-31	GATA-3	CCR3 CCR4 CCR8 CXCR4 IL-17RB	STAT5 STAT6	IL-4

*proposed to represent a distinct T helper (Th_9) subset

Th_{17} subset

IL-17 was originally classified as Th_1-associated cytokine before studies showed that $IFN\gamma^+$ and $IL\text{-}17^+$ T cells were divergently regulated (Oppmann *et al* 2000, Cua *et al* 2003) and occurred as two separate subsets *in vivo* (Langrish *et al* 2005, Park *et al* 2005, Harrington *et al* 2005). Today, Th_{17} cells are a widely accepted T helper subset with distinct functions from Th_1 and Th_2 cells. Their expression profile has, since, expanded to not only include IL-17A and IL-17F but also IL-21, IL-22, and IL-26 (Wilson *et al* 2007, Dambacher *et al* 2009).

Th_{17} differentiation

Th_{17} cell development was dependent on IL-6 and Transforming growth factor β1(TGFβ1; Veldhoen *et al* 2006, Bertelli *et al* 2006, Mangan *et al* 2006). While the presence of TGFβ1 was important (Li *et al* 2007, Manel *et al* 2008), IL-6 appeared to be pivotal in determining Th_{17} rather than T regulatory cell development (Zhou *et al* 2006). Other cytokines which have, since, been shown to be important include IL-1β (Acosta-Rodriguez *et al* 2007, Wilson *et al* 2007), IL-21 (Korn *et al* 2007, Nurieva *et al* 2007) as well as IL-23 (McGeachy *et al* 2007, Korn *et al* 2009).

Transcriptional regulation

The transcription factor Rar related orphan nuclear receptor C 2 (RORC2; in mice: RORγt) was shown to be essential for controlling IL-17 expression and Th_{17} development (Ivanov *et al* 2006, Zhang *et al* 2008). While RORγt/RORC2 is often quoted as the master regulator of Th_{17} cells, Interferon regulating factor 4 (IRF4) has shown to be essential for IL-17 expression as well (Brustle *et al* 2007).

Th_{17} cells show strong STAT3 expression which is essential for Th_{17} development (Zhou *et al* Spolski *et al* 2007, Nurieva *et al* 2007) e.g. down regulating FOXP3 (Korn *et al* 2007) and Th_{17} cell function (Xang *et al* 2007, Nishihara *et al* 2007) binding directly to the IL-17 and IL-21 promoter (Wei *et al* 2007, Chen *et al* 2006). An overview of the Th_{17} cell classifications is given in the table below (Table 6).

Table 6. Th_{17} subset classification

Subset	Lead Cytokine(s)	Assoc. cytokines	Transcription Factors	Surface Expression	Stat regulation	Polarising cytokines
Th_{17}	IL-17A[1] IL-17F	IL-21 IL-22[12] IL-26	RORC2[1] IRF4	CCR6[1] IL-1R1 IL-12RB IL-23R[1] CD161[2] IL-13Ra1	STAT3[3]	TGFβ1[4] IL-1β IL-6 IL-21[5] IL-23

Based on ([1]Annunziato Cosmi 2007,[2]Cosmi De Palma 2008, [3]Nishihara Ogura 2007, [4]Veldhoen Stockinger 2006, [5]Korn 2007) induces IL-22 during Th_{17} differentiation (McGeachy 2007)

Regulatory T cells

A hallmark of the adaptive immune system is the regulation of the inflammatory response through tolerance induction. CD4 T cells can exhibit regulatory properties and form an integral part of the immune regulation within the body (Sakaguchi *et al* 1995). Regulatory CD4 T cells, themselves, represent a diverse group of cells. They include thymus-derived natural T regulatory cells as well as inducible T regulatory cells (Ito *et al* 2008). The mechanism in which they induce tolerance is by contact dependent (e.g. CTLA-4, OX40, ICOS) as well as contact independent means (e.g. IL-10, TGFβ1) (Shevach *et al* 2009). As regulatory T cells themselves represent a large and heterogeneous group, individual sub-classifications have been proposed (e.g. IL-10 producing Tr_1 and TGFβ1 producing Th_3 cells, Groux *et al* 1997, Chen *et al* 1994).

Treg differentiation

The induction of T regulatory cells is influenced by the strength of TCR engagement (Bautista *et al* 2009, Leung *et al* 2009), the binding of co-stimulation molecules (e.g. CD28, CD80/86; Salomon *et al* 2000, Tai *et al* 2005) as well as the soluble factors expressed during activation (e.g. IL-2, TGFβ1; Fontenot *et al* 2005, Kretschmer *et al* 2005).

Transcriptional regulation

While not all regulatory T cells express FOXP3 (e.g. Tr1 cells), the transcription factor is essential for T regulatory cell development and function (Fontenot *et al* 2003, Fontenot *et al* 2005). Similarly, STAT5 expression activated by the presence of IL-2 was shown to bind to FOXP3 promoter and enhance its expression (Davidson *et al* 2007, Burchill *et al* 2007). An overview of the T regulatory cell classification is given in the table below (Table 7).

Table 7. T regulatory subset classification

Subset	Lead Cytokine	Assoc. Cytokines	Master Regulator	Surface Expression	Stat regulation	Polarising cytokines
Tregs	IL-10 or TGFβ1		FOXP3	$CD25^{high}$	STAT5	TGFβ1 IL-2

The novel Th_{22} subset

IL-22, itself, was known to be expressed by CD4 T cells (Dumoutier *et al* 2000) and, early on, was classified as a Th_1 rather than Th_2 associated cytokine (Gurney *et al* 2004). With the growing acceptance of new Th_{17} subset, IL-22 was re-classified to be a Th_{17}-associated cytokine (Liang *et al* 2006, Chung *et al* 2006). Not long after its re-classification, it became apparent that the expression of IL-22 and IL-17 were controlled by differential regulatory pathways (Zheng *et al* 2007) and could be largely observed as distinct IL-17^+ and IL-22^+ T cell populations *in vivo* (Scriba *et al* 2008). These observations questioned the new Th_{17} association and led to the hypothesis that IL-17^+ and IL-22^+ T cells should be viewed as two distinct Th_{17} and Th_{22} subsets (Duhen *et al* 2009, Eyerich *et al* 2009, Nograles *et al* 2009, Trifani and Spitz 2010).

Th_{22} expression profile

The consensus is that Th_{22} cells express IL-22 in the absence of IFNγ, IL-4 and IL-17. However, this research group identified that memory Th_{22} cells derived from skin were always able to co-express the Th_1-associated cytokine TNFα (Eyerich *et al* 2009). In addition, the research highlighted a selective expression of growth factor genes such as fibroblast growth factors (Eyerich *et al* 2009).

An initial survey of chemokine receptor expression profile showed that CCR4 (Trifani and Spitz 2010, Duhen *et al* 2009), CCR6 (Trifani and Spitz 2010, Duhen *et al* 2009) and CCR10 (Trifani and Spitz 2010, Duhen *et al* 2009, Eyerich *et al* 2009) were preferentially expressed on Th_{22} cells. While their selective enrichment of Th_{22} cells remained poor, other receptors identified to be Th_{22} specific (e.g. PDGFR, FGFR and TIE1; Eyerich *et al* 2009) still need to be verified.

Th_{22} differentiation

Research showed that IL-22^+ IL-17^- CD4 T cells could be primed by APC including langerhans cells, dermal dendritic cells as well as plasmacytoid dendritic cells *in vitro* (Fujita *et al* 2009, Duhen *et al* 2009). All of these cells were able to induce IL-22 expression in the absence of additional antigen mediator stimulation (Fujita *et al* 2009, Duhen *et al* 2009). Yet, the maturation of plasmacytoid dendritic cells in CpG rather than LPS containing medium increased their ability to prime IL-22^+ T cells (Duhen *et al* 2009).

The T cell priming is dependent on both signals relayed via cell-cell contact (e.g. surface molecules) as well as soluble mediators (e.g. cytokines). Contact dependent signals which

were shown to be important include T cell receptor (TCR), CD28, ICAM1 (Gurney *et al* 2004) as well as CRTAM engagement (Yeh *et al* 2008). The importance of CRTAM was highlighted using a CRTAM -/- knockout mice which had strongly reduced IL-22 (and IFNγ) levels while IL-17A expression remained unaffected (Yeh *et al* 2008).

Soluble mediators important in priming of Th_{22} cells were IL-6 and TNFα as well as IL-23. IL-6 and TNFα have never been linked to IL-22 expression before but their combination was recently shown to drive *in vitro* Th_{22} differentiation in the absence of APC stimulation (Duhen *et al* 2009). IL-23, on the other hand, is known to be important for IL-22 expression *in vitro* and *in vivo* (Zheng *et al* 2007, Chung *et al* et al 2006, Kreymborg *et al* 2007, Volpe *et al* 2009). While the IL-23 receptor is not found on naive T cells, it is believed to be induced after T cell activation (Wilson *et al* 2007) and APC were shown to be a major source of early IL-23 (Siegemund *et al* 2009). The dependence on IL-23 signalling was proven using both IL-23 blocking antibodies (Liang *et al* 2007) as well as knockout mice (Aujla *et al* 2008, Zheng *et al* 2008, Godinez *et al* 2009, Siegemund *et al* 2009, de Luca *et al* 2010). In all studies, IL-22 expression was reduced to either small or undetectable levels. Interestingly, other IL-23 induced cytokines such as IL-1β, IL-6, and IL-17A were not as severely affected suggesting that there is little or no redundancy in the induction of IL-22 (Kleinscheck *et al* 2010). In addition, other factors influencing the expression of IL-22 in CD4 T cells are listed in the table below (Table 8).

Table 8. Factors influencing the expression of IL-22 in CD4 T cells

Mediator	+/-	Function	Reference
IL-23	+	Induction of IL-22	Zheng *et al* 2007, Chung, Yang *et al* 2006
TNFα + IL-6	+	Induction of IL-22 but not IL-17	Duhen *et al* 2009
IL-1β	+	Synergy with IL-23	Liu *et al* 2008, Liang *et al* 2006, Kleinscheck *et al* 2009
IFNγ	+	may block IL-17a but not IL-22 induction	Scriba *et al* 2008
IL-2	+	May block IL-17a but not IL-22 induction	Veldhoen *et al* 2009
IL-12p40	+	May help IL-22 induction	Kreymborg *et al* 2007
1,25(OH)2D3	+	Induction of IL-23 receptor	Ikeda *et al* 2010
1,25(OH)2D3	-	Blockade of IL-22, IL-17a and IFNγ	Colin *et al* 2010
IL-4	-	Blockade of IL-22 and IL-17a	Zheng *et al* 2007, Veldhoen *et al* 2009, Schnyder *et al* 2010
IL-10	-	Decrease in IL-22	Gu *et al* 2008
IL-12p35 (IL-12/IL35)	+/-	No role in IL-22 induction/inhibition	Siegemund *et al* 2009
IL-17a	-	Weakly inhibited IL-22	Sonnenberg *et al* 2010, de Luca *et al* 2010
PGE2	-	Blockade of IL-22 induction of IL-17a	Chizzolini *et al* 2008
EBI3 (IL27/Il35)	-	Blockade of IL-22 and IL-17a	Yang *et al* 2008
Collagen V	-	Reduction of IL-22 and IL-17a	Brown *et al* 2010

Transcriptional regulation

IL-22 expression is dependent on Notch (Alam *et al* 2010) as well as aryl hydrocarbon receptor (AHR) signalling (Velhoen *et al* 2008 & 2009). The importance of AHR signalling

was demonstrated using artificial AHR agonists (e.g. bNF and FICZ) which increased IL-22 expression in CD4 T cells (Veldhoen *et al* 2008) while decreasing IL-17A (Trifani *et al* 2010). While the knock down of AHR by siRNA showed only a small reduction in IL-22 expression, (Trifani *et al* 2010) the AHR -/- knock out mouse was unable to mount an IL-22 response (Veldhoen *et al* 2008 & 2009). Due to its strong connection to IL-22, AHR is often quoted as the master regulator of Th_{22} cells. However, AHR is also essential for the development of FOXP3 negative IL-10^+ T cells (Mezrich *et al* 2010, Apetoh *et al* 2010, Gandhi *et al* 2010) and the essential factors in regulating Th_{22} cell development, therefore, remain to be explored. An overview of the proposed Th_{22} cell classifications is given in the table below (Table 9).

Table 9. Th_{22} subset classification

Subset	Lead Cytokine	Assoc. Cytokines	Master Regulator	Surface Expression	Stat regulation	Polarising cytokines
Th_{22}	IL-22[1,2,4,5]	TNFα[1]	AHR[3]	CCR4[2,4] CCR6[2,4] CCR10[1,2,4] PDGFR FGFR2 TIE1*		IL-6[2] TNFα[2]

Based on ([1]Eyerich 2009, [2]Duhen 2009, [3]Velhoen 2008, [4]Trifani 2009, [5]Nograles 2009,* Eyerich & Schmidt-Weber unpublished)

T helper subset heterogeneity and plasticity

The original definition of CD4 T cells into Th_1 and Th_2 T helper subsets has advanced our understanding of T cell biology significantly. As more evidence emerges, it becomes apparent that T helper cell priming may not necessarily lead to terminal differentiation and confer a stable and distinct T helper subset identity.

In fact, it is likely that CD4 T cells retain some degree of plasticity after activation allowing different signals within the inflamed tissue to alter their expression profile. This is supported by observations showing the partial or full conversion of Th_2 -> Th_1 (Fujimura *et al* 1997) as well as Th_1 <-> Th_{17} <-> Treg *in vitro* and *in vivo* (Annunziato *et al* 2007, Osorio *et al* 2008, Martin-Orozco *et al* 2009). Not just the stability of T helper cell identities is questioned but also the definition of a distinct T helper subset. The ever more detailed delineation of existing T helper subsets such as Th_2 -> Th_9 and Th_{17} -> Th_{22}, in particular, highlights the need to study T helper cell lineages in a more comprehensive way. While large scale epigenetic studies are under way to re-define T helper cell fate development (Wei *et al* 2009, Placek *et al* 2009, Wilson *et al* 2009), our current model of T helper subsets and their definitions remain in place.

Interleukin-22

IL-22

Gene and Protein

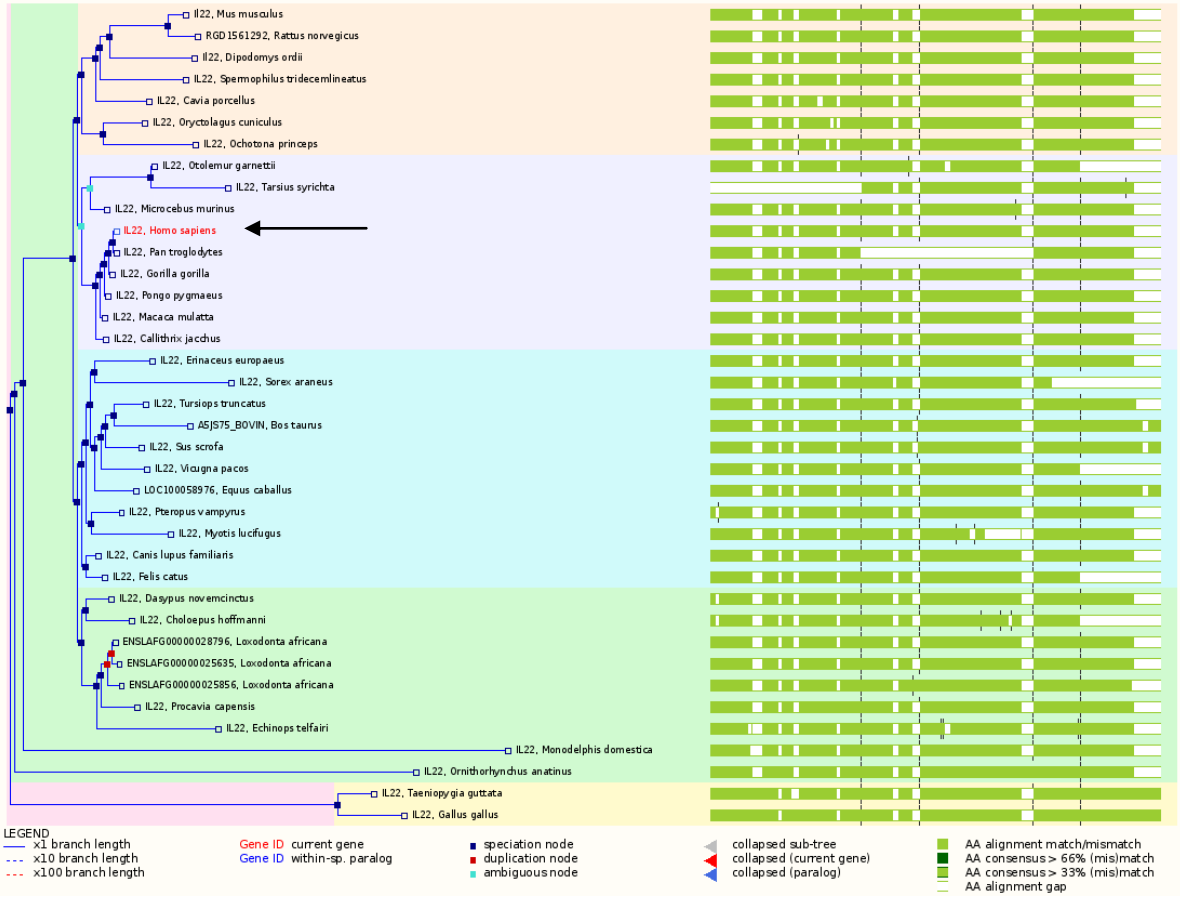


Figure 1. Genomic relationship between all species with known IL-22 genes (source: www.esembl.org)

The Interleukin 22 gene is an evolutionary conserved gene that appears in most parts of the animal kingdom including birds (e.g. chicken, zebra finch), fish (e.g. zebra fish), mammals, marsupials, primates, rodents and cetaceans (e.g. dolphin) but not reptiles or amphibians (Figure 1). IL-22 may have arisen at the same time when antigen specificity (occurrence of RAG genes) and the adaptive immunity developed and is induced after Lipopolysaccharide (LPS) encounter across species form fish to man (Igawa *et al* 2006).

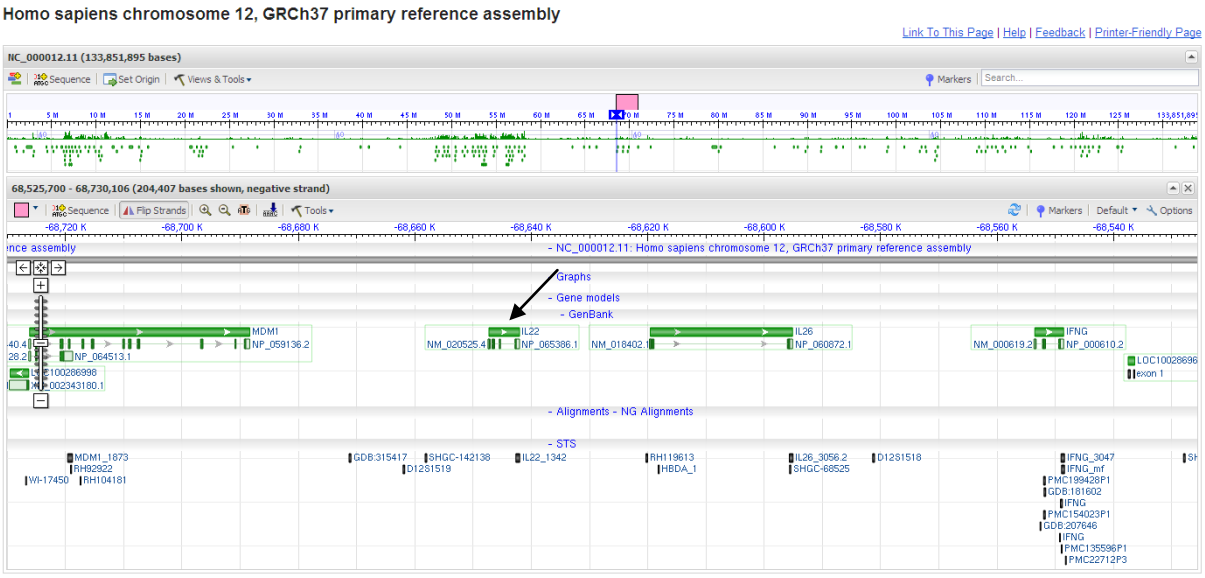


Figure 2. Genomic location of the IL-22 gene (source: www.ncbi.nlm.nih.gov)

The human IL-22 gene is found near the IL-26 (AK155) gene and, in close proximity, to the Mdm4, transformed 3T3 cell double minute 1, p53 binding protein (MDM1) and IFNγ genes (Figure 2). The genomic region is frequently found under the name 12q15 and has been under extensive research in many genome wide disease association studies (e.g. 12q15 association with asthma, Barnes *et al* 1996). The IL-22 and IL-26 genes show some limited homology with IL-10 (10-27%; Kotenko *et al* 2001) and have, thus, both been classified as IL-10-related cytokines. More modern analysis has shown that IL-22 is more closely related to IFN-λ and, in particular, IFN-λ3 (Gad *et al* 2009). Yet, studies remained inconclusive about the functional relationship between IL-22 and IL-10 (Lecart *et al* 2002, Wolk *et al* 2005) as well as IL-22 and IFNλ (Pagliaccetti *et al* 2010).

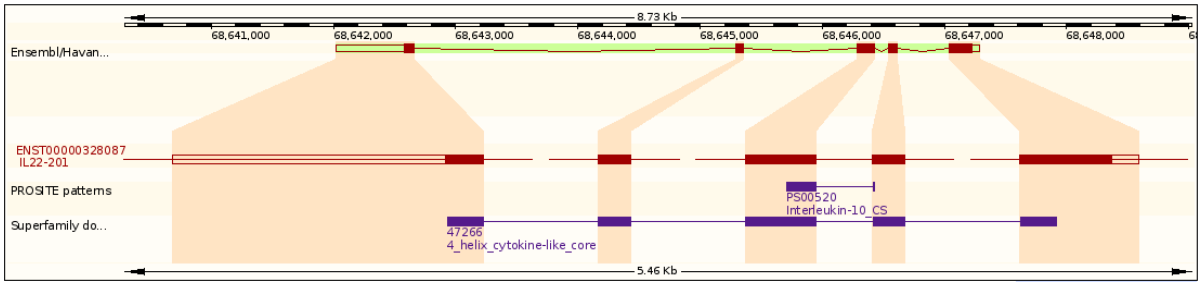


Figure 3. Overview of the IL-22 gene and its mRNA transcript (source: www.ensembl.org)

The IL-22 gene has five exons (Figure 3) and is transcribed into a single mRNA (NM_020525). While in humans only one gene copy exist, several strains of mice were shown to have a second IL-22 gene copy which, likely, arose during gene duplication and is classified as a pseudogene (B10.S, SWR, C57Bl/6, FVB, 129/Sv, C3H, DBA/1; Levillayer *et al* 2007).

The IL-22 core promoter region is believed to be 641 nucleotides upstream and includes the first IL-22 exon (Dumoutier *et al* 2000b). This presumed IL-22 promoter region has no TATAAT motif, which is not uncommon, as only about 50% of genes are known to be TATA box regulated (Lewin, Genex IX 2008). Today, however, analysing gene promoter regions would include wider genomic regions. This is due to the fact that many important promoter, enhancer and silencer regions have been found several thousand nucleotides upstream or downstream of the start codon (Lewin, Genex IX 2008). A more recent computer guided analysis and gel shift assay of the bovine IL-22 gene showed that the -1132 and -879 region in the 5' upstream gene sequence was a likely promoter site contained putative transcription factor binding sites for STATx, Sox-5/9, Sp1, Ik-1, and AREB6 (Ma *et al* 2010).

IL-22 Protein

The IL-22 mRNA is transcribed into a single 179 amino acid long protein (NP_065386.1). It contains 6 α-helices which are arranged in anti-parallel conformation and result in a monomeric, bundle-like protein (Diagram 2; Nagem *et al* 2002, Xu *et al* 2005). The monomeric conformation of crystallised IL-22, at least at physiologically relevant concentrations, was confirmed by gel filtration chromatography and dynamic light scattering studies (Nagem *et al* 2002, Logsdon *et al* 2002). The IL-22 primary structure contains four Cys which form two intramolecular disulfide bridge bonds (Cys40-Cys132 linking the N terminus to the DE loop and Cys89-Cys178 linking helix C to helix F; Nagem *et al* 2002). IL-22 is glycosylated on helix A (Asn54-Arg55-Thr56), in the AB loop (Asn68-Asn69-Thr70) and in helix C (Asn97-Phe98-Thr99) of which the glycosylation on Asn54 in helix A has been found to be essential for the interaction with IL-10R2 (Logsdon *et al* 2004).

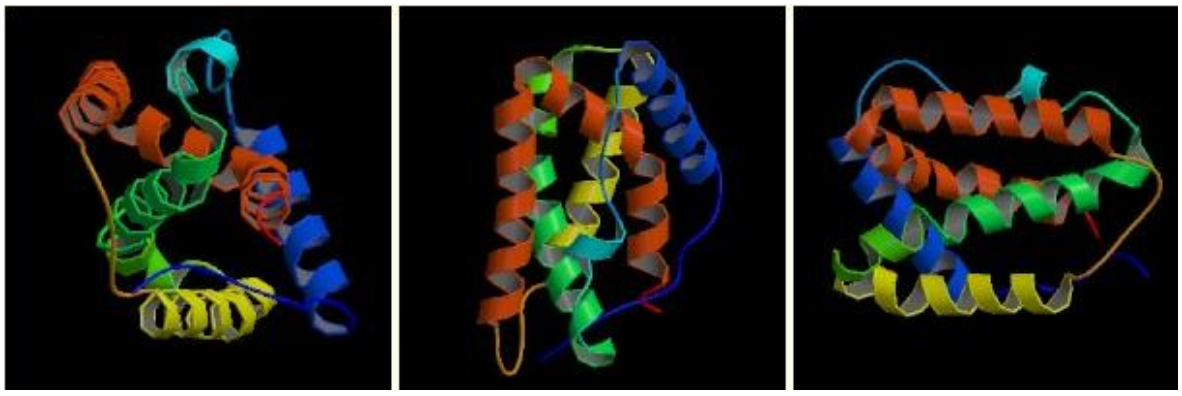


Diagram 2. Predicted 3-dimensional structure of IL-22 (http:// modbase.compbio.ucsf.edu/)

Expression

Interleukin 22 is a mediator exclusively produced and secreted by cells of the immune system. Originally believed to be released only by CD4 T cells, depletion studies of innate and adaptive T cells as well as NK cells showed that IL-22 could be expressed by a wider range of cell types (Hedrick *et al* 2009, Munoz *et al* 2009, de Luca *et al* 2010, van Maele *et al* 2010). Today, three broad categories of cell types have been identified to be capable of producing IL-22. These include the myeloid-derived cells (monocyte/ macrophage/ dendritic cell), the NK/ LTi/ LTi-like cells and the innate/ adaptive T lymphocyte populations.

Innate Immune cells

Monocytes, macrophages and dendritic cells

Evidence that IL-22 is produced by myeloid cells has become more substantial over the years. The earliest description of monocytes to express IL-22 came from a study investigating gene expression profiles of adherent PBMCs after bypass surgery (Hsing *et al* 2006). These cells expressed high levels of IL-22 but also IL-19 and IL-20 mRNA. More direct evidence of IL-22 expression was obtained by culturing monocytes in IL-23 or LPS containing media (Zheng *et al* 2008). *In vivo* macrophages identified by $CD11b^+MAC3^+$ or $CD11b^+CD86^+$ were shown to be able to secrete IL-22 as well (Kapessidou *et al* 2008, Gu *et al* 2008). $CD11b^+$ and $CD11c^+$ cells which could represent either macrophages or dendritic cells showed to express the IL-23 receptor and respond similarly to IL-23 challenge with IL-22 release (Awasthi *et al* 2009). $CD11c^+$ cells generally categorised specifically as dendritic cells were found to produce IL-22 in several studies (Zheng *et al* 2007 & 2008, Pickert *et al* 2008). While depletion of T cells has shown that monocytes, macrophages and dendrtic cells only produce small amounts of IL-22 *in vivo*, their IL-22 expression capacity may still be important on a local cell to cell level.

Lymphoid-Tissue-inducer (LTi), LTi-like, and NK cells

Several studies have shown that IL-22 together with IFNγ is essential effector cytokine of Lymphoid tissue inducer (LTi) cells (Satoh-Takayama *et al* 2008, Luci *et al* 2009, Cupedo *et al* 2009). LTi cells developed, in parallel, with the adaptive immune response facilitate the association of $CD4^+$ T cells with dendritic cells in several species (e.g. fish, birds) in the absence of secondary lymphoid tissue and even, in humans, are thought to still facilitate interactions between $CD4^+$ T cells and dendritic cells within the submucosa (Lane *et al* 2008). Indeed, in the gut submucosa, LTi cells have been found in close contact with $CD1c^+$ dendritic cells and $CD4^+$ T cells. Through their expression of LTαβ2, they interact with stromal cells which, in turn, start to secrete and attract B cells (through CXCL13), T cells and dendritic ells (through CCL21, CCL19). LTi cells express OX40-ligand and CD30L which act as survival signals for $CD4^+$ T cells thus maintain peripheral T cell memory. In addition, LTi

cells were shown to differentiate into $CD11c^+$ dendritic cells under IL-3 and TNFα conditions. However, LTi cell numbers both in mice and human are very high. This suggests that they may have acquired additional immune functions. The identity of LTi cells is not always easily established as they can express many of the NK cell associated surface receptors (NKp44/46, CD56) leading to some misclassification between the two cell populations.

Classic Natural killer (NK) cells have shown to be IL-22 positive as well. NK cells utilize their surface receptor repertoire to discriminate between healthy and infected or abnormal cells and cause their destruction. In an immature state, they produce only cytokines like IFNγ ($CD56^{bright}$) while, in a mature state, they can produce perforin and granzyme ($CD56^{dim}$). IL-22 production in NK cells was identified very early on (Wolk *et al* 2002 & 2004) but was, then, believed not to be of any significance. However, this view changed significantly as many NK cells in the tissue have, now, been found to produce IL-22 at the front line of mucosal defences. This led to the classification of so called NK_{22} cells.

However, their close anatomical location to $IL\text{-}22^+$ LTi cells and the shared expression of NK cell surface markers by both cell types has questioned the true identity of these innate $IL\text{-}22^+$ cells. Several years ago, it was shown than LTi cells can convert into classical NK cells demonstrating a developmental relationship between both cell types (Mebius *et al* 1997). For the moment, however, NK and LTi cells are still being treated separately in the literature. One discriminating feature between NK cells and LTi cells is the dependency on IL-15 or IL-7 respectively. Therefore, IL-7α receptor expression would suggest an LTi-associated development path while the dependency on IL-15 would suggest that of NK cells. In addition, IL-22 producing cells appear to be RORc positive. RORc is required for LTi development but is not essential for NK cell formation.

For both NK (Cella *et al* 2009, Guo *et al* 2010, Dhiman *et al* 2009, Satoh-Takayama *et al* 2009) and LTi (Takatori *et al* 2009) cell populations, IL-23 appears to be the essential trigger for IL-22 release. In addition, IL-1β (Cella *et al* 2009, Hughes *et al* 2010), IL-15 (Dhiman *et al* 2009) and IL-18 (Satok-Takayama *et al* 2009) have been shown to trigger IL-22 release in NK cells while, in LTi cells, IL-15 down regulated IL-22 expression (Kim *et al* 2010). An overview of the current evidence of $IL\text{-}22^+$ NK, LTi, and LTi-like cell populations is given in the table below (Table 10).

Table 10. IL-22 expressing NK, LTi, and LTi-like cell populations

Classified as	Discriminating factor	Species	Reference
NK (IL-22+)	NKp44+ cells	Human	Crellin *et al* 2010
	NKp44+CD56+	Human	Cella *et al* 2009
	NK1.1+CD27-	Mouse	Guo *et al* 2010
	NK1.1+CD3-	Mouse	Veldhoen *et al* 2009
	CD3-CD56-	Human	Dhiman *et al* 2009
	CD117+CD161+	Human	Hughes *et al* 2009 & 2010
	RORγt+NKp46+	Mouse	Luci *et al* 2009
LTi/-like (IL-22+)	CD127+RORC+	Human	Crellin *et al* 2010
	CD56+CD127+RORC+NKp46+	Human	Crelin *et al* 2010
	CD117+CD3-	Human	Satoh-Takayama *et al* 2008, Luci *et al* 2009
	CD3-CD19-CD14-CD127+RORC+	Human	Cupedo *et al* 2009
	CD4+CD3-NK1.1-CD11b-Gr1-CD11c-B220-	Mouse	Takatori *et al* 2009
	CD56+CD127+	Human	Cupedo *et al* 2009
	NKp46+RORgt+	Mouse	Sanos *et al* 2009

Innate T lymphocytes

Innate T lymphocytes are important first line defences in mucosal surfaces but can only recognise a limited antigen repertoire. γδT cells (Zheng *et al* 2007, Yao *et al* 2010, Ness-Schwickerath *et al* 2010, Ma *et al* 2010, Sutton e*t al* 2009, Martin *et al* 2009), iNKT and NKT cells (Wahl *et al* 2009, Goto *et al* 2009, Ma *et al* 2009, Sutton *et al* 2009, Martin *et al* 2009) were shown to be IL-22^{+} *in vitro* and *in vivo*.

Of these cells, γδT cells are best researched and were shown to produce IL-22 after IL-23 (Zheng *et al* 2007, Sutton *et al* 2009), IL-1β (Sutton *et al* 2009), AHR, TLR-2, and Dectin-1 agonist challenge (Martin *et al* 2009). Down regulation of IL-22 was seen when γδT cells were stimulated with phosphoantigen (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) which is produced by some mycobacteria and opportunistic pathogens (Yao *et al* 2010). In humans, IL-22 production by γδT cells can be significant and account for up to 50% of T cell-released IL-22 (Ness-Schwickerath *et al* 2010).

Adaptive Immune cells

Adaptive Immune cells are divided into T and B lymphocytes but, until now, B cells have never been identified to produce IL-22. Adaptive T cells are further classified according to their MHC class recognition. CD8 T cells recognise MHC class I and CD4 T cells recognise MHC class II.

CD4^{+} T lymphocytes

CD4^{+} T cells are believed to be the major source of IL-22 in humans and IL-22 expression has, so far, been identified in several different T helper subsets (see chapter T helper subsets; page 34).

CD8$^+$ T lymphocytes

In humans, CD8$^+$ T cells were shown to contribute to the overall IL-22 release (Zheng *et al* 2007, Hamada *et al* 2009). Their selective expression of IL-22 was dependent on IL-23 lead to a similar classification of a Tc_{22} subset of CD8$^+$ T cells (Scriba *et al* 2008, Sellge *et al* 2010).

IL-22 Receptors

IL-22RA1

Gene and Protein

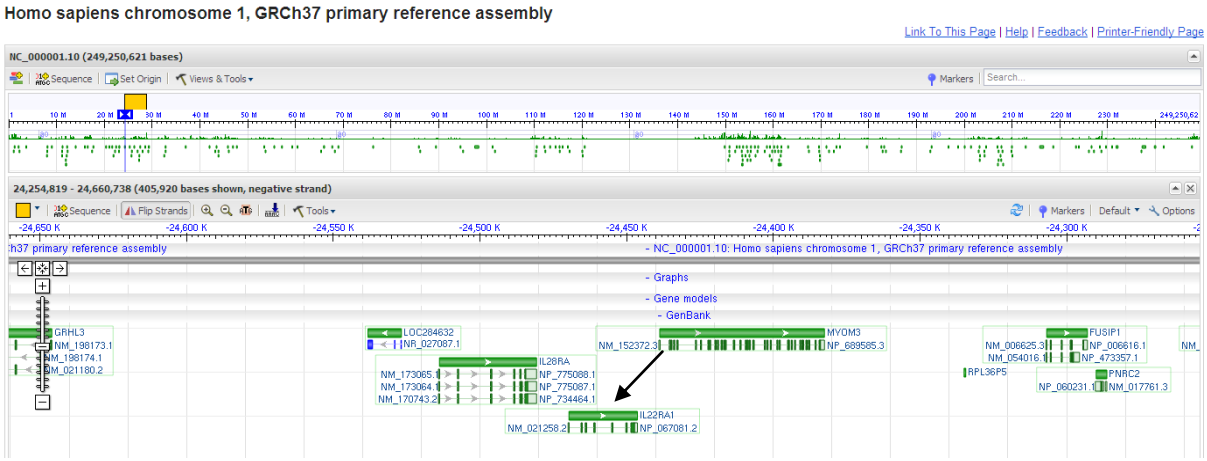


Figure 4. Genomic location of the IL-22RA1 gene (http://www.ncbi.nlm.nih.gov/nuccore/NC_000001.10?from=24445094&to=24470778&strand=true&report=graph)

The IL-22RA1 gene is classified as Interferon receptor related having interferon receptor activity (source: amigo.geneontology.org). In humans, the IL-22RA1 gene is located on chromosome 1 (NC_000001.10) between 24446261 and 24469611 of the complement strand in close proximity to the IL28RA and MYOM3 genes (Figure 4). The gene encodes 7 exons and is transcribed into a single mRNA (NM_021258.2) and translated into a single receptor precursor (NP_067081.2).

Function - IL-22 signalling complex

IL-22RA1 can form two signalling complexes either with IL-10RB for IL-22 signalling or IL-20RA2 for IL-24 signalling. IL-22 signalling was shown to activate ERK1/2, pSAPK, JNK (Xie *et al* 2000, Brand *et al* 2006 & 2007), JAK (Xie *et al* 2000), pAKT (Zenewicz *et al* 2008) and STAT 1,2,3, and 5 (Xie *et al* 2000, Brand *et al* 2006 & 2007, Wolk *et al* 2007, Dumoutier *et al* 2009). STAT3, in particular, has been shown to become phosphorylated and, together with a chimeric STAT3/STAT5 protein, was constitutively associated with the C-terminal of the IL-22RA1 receptor (Dumoutier *et al* 2009). Another direct link in the IL-22RA1 signal

transduction is Shp2 which is essential for IL-22 induced MAP kinase activation (Meng *et al* 2010).

Expression

Immune system

Comprehensive early studies found no evidence that IL-22RA1 was expressed by either B cells, T cells, monocytes, macrophages, dendritic cells or NK cells (Kunz *et al* 2006, Wolk *et al* 2008). All other evidence linking IL-22RA1 to immune cells has remained indirect (Table 11).

Table 11. Evidence of IL-22RA1 expression in immune cells

Type	Specific Cell	Evidence
Immune cells	Monocytes/Neutrophils	Indirect evidence – $CD66^{+}IL\text{-}22RA1^{+}$ cells in synovial fluid (Kragstrup 2008)
	Macrophages	Indirect evidence – responsive to IL-22 *in vitro* (Dhiman *et al* 2009)
	Dendritic cells	Indirect evidence – responsive to IL-22 *in vitro* (Schnyder *et al* 2010)

Structural cells

IL-22RA1 expression shows a strong correlation to structural cells and, in particular, those of epithelial origin (Table 12). In addition, IL-22RA1 is expressed by specialised cells such as hepatocytes in the liver and islet cells in the pancreas. The expression of IL-22RA1 in other cell types such as endothelial cells, muscle cells, and fibroblasts is less well documented and remains controversial.

Table 12: IL-22RA1 expression in human structural cells

Tissue	Specific Cell type	Evidence
Epithelial cells	Keratinocytes	6% IL-22RA1 surface expression in culture (Kragstrup *et al* 2008), IL-22RA1 mRNA increase afte$TNF\alpha$ (Wolk *et al* 2009) and $IFN\gamma$ (Kunz *et al* 2006) exposure
	Bronchial epithelial cells	15% IL-22RA1 surface expression in culture (Aujla *et al* 2008), mRNA and protein (Kotenko *et al* 2001,Ramanathan *et al* 2007, Aujla *et al* 2008)
	Sinosinal bronchial epithelial cells	IL-22RA1 mRNA and protein (Kotenko *et al* 2001, Ramanathan *et al* 2007)
	Intestinal epithelial cells	Increased IL-22RA1 after LPS, IL-1β, and $TNF\alpha$ exposure (Brand *et al* 2006)
	Retinal pigment epithelial cells	IL-22RA1 protein (Li *et al* 2008)
Endothelial cells	Blood-brain barrier cells ($caveolin\text{-}1^{+}$)	16% IL-22RA surface expression in culture (Kebir *et al* 2007)
Fibroblasts	Synovial fibroblasts ($vimentin^{+}$)	IL-22RA1 protein (Ikeuchi *et al* 2005)
	Skin fibroblast	IL-22RA1 protein (Wolk *et al* 2009)
Muscle cells	Skeletal muscle	IL-22RA1 mRNA (Kotenko *et al* 2001)
	Heart muscle	IL-22RA1 mRNA (Kotenko *et al* 2001, Wolk *et al* 2004)
Specialised cells	Pancreatic islets of Langerhans	IL-22RA1 mRNA (Aggarwal *et al* 2001)
	Hepatocytes	IL-22RA1 mRNA and protein (Aggarwal *et al* 2001, Kotenko *et al* 2001, Wolk *et al* 2005, Brand *et al* 2006, Wolk *et al* 2007, Dambacher *et al* 2008)

IL-22RA2

Gene and Protein

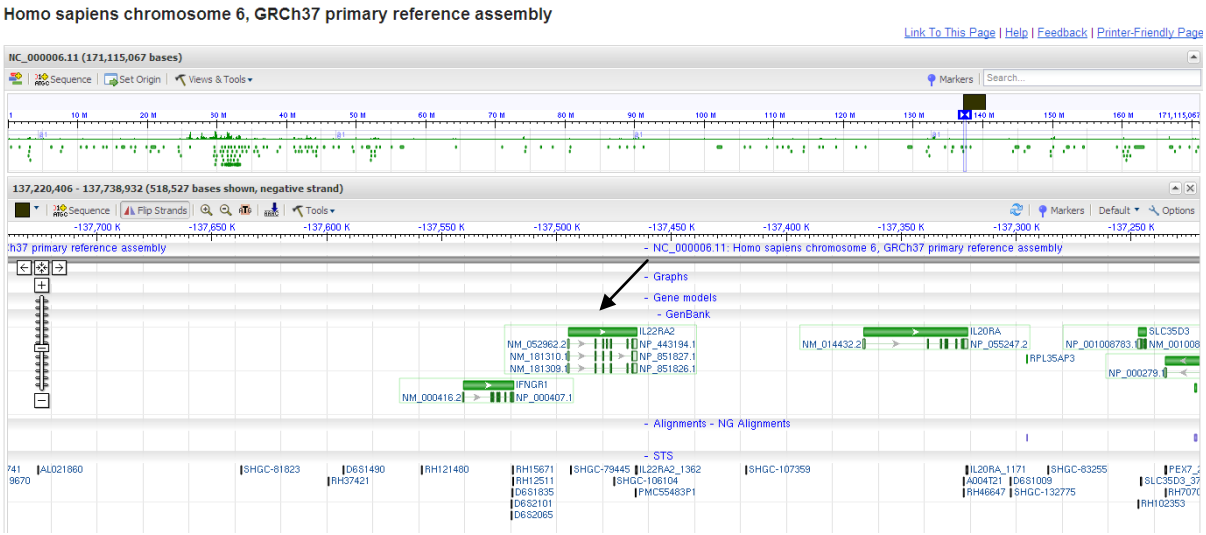


Figure 5. Genomic location of the IL-22RA2 gene (http://www.ncbi.nlm.nih.gov/nuccore/NC_000006.11?from=137463466&to=137496276&strand=true&report=graph)

The Interleukin 22 Receptor alpha two gene (IL-22RA2) is found across all IL-22 expressing species suggesting that it may have evolved at a similar point in time and was retained throughout evolution indicating a selective advantage. In humans, the IL-22RA2 gene is located on chromosome 6 (NC_000006.11) between 137464957 and 137494785 of the complement strand. It is in close proximity to the IFNGR1 gene and evolutionary, functionally and closely related IL-20RA gene (Figure 5).

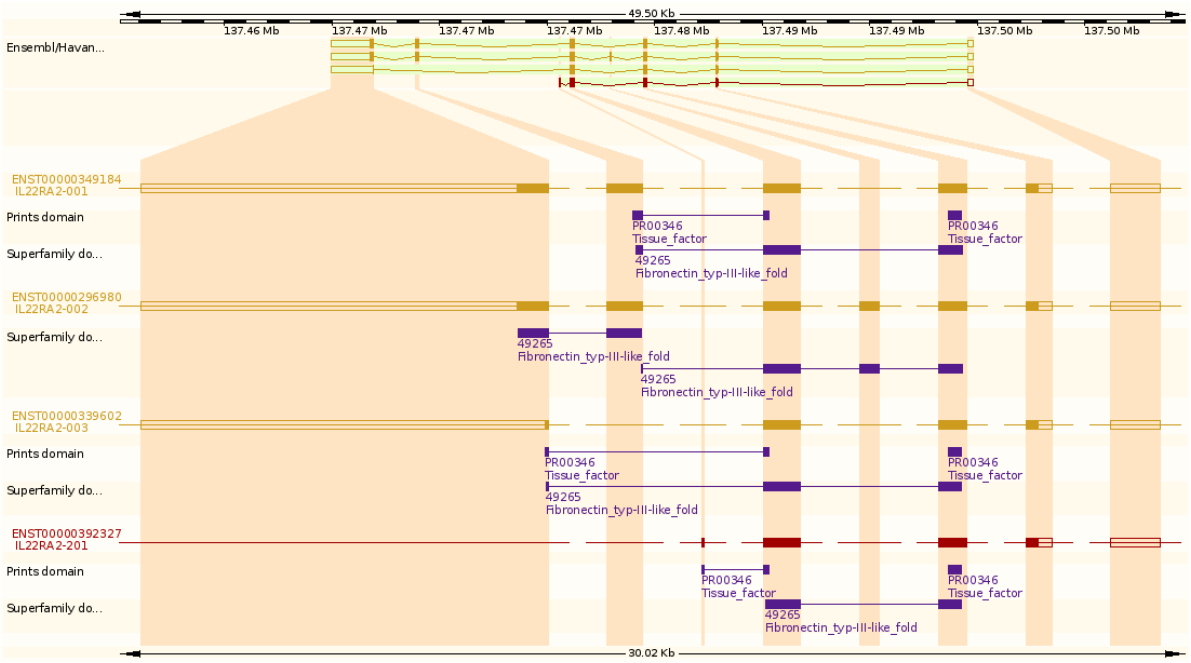


Figure 6. Overview of the IL-22RA2 gene and its mRNA transcripts (source: www.ensembl.org)

The IL-22RA2 gene is translated into 4 distinct mRNA's of which all are protein coding. The longest transcript IL-22RA2-002 includes a 7th exon and is translated into a 263 amino acid long protein. The 7th exon in the transcript may be a relatively recent addition in the history of the IL-22RA2 gene that occurred prior to the divergence of Apes and Old World monkeys by the action of retrotransposon (Piriyapongsa *et al* 2007). This longest IL-22RA2 protein failed to bind IL-22 and it is, yet, unknown if the protein has a ligand partner or is non-functional (Dumoutier *et al* 2001, Wolk *et al* 2007). Both the IL-22RA2-003 and very short IL-22RA2-201 mRNAs are translated but too may be non-functional (Kotenko *et al* 2001). The second longest transcript IL-22RA2-001 (NM_181309) is translated into a 231 amino acid long protein (NP_851826.1). This protein does bind IL-22 (Dumoutier *et al* 2001a &2001b, Kotenko *et al* 2001, Xu *et al* 2001) and is generally considered when speaking about IL-22RA2.

Function - IL-22 agonist

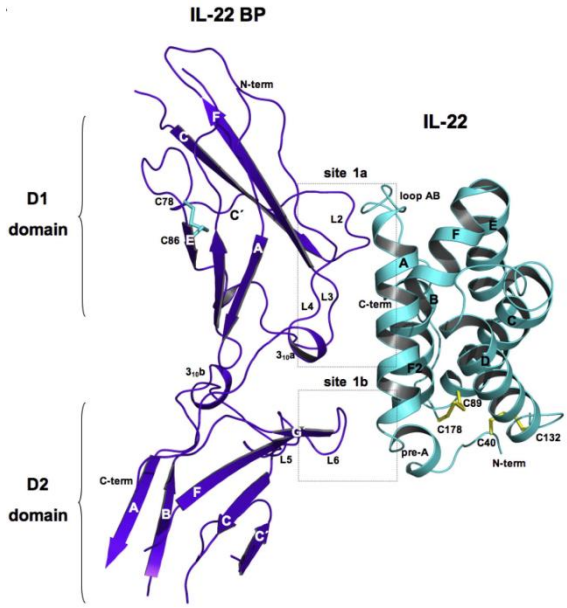


Diagram 3. The 3-dimensional configuration of IL-22RA2 binding to IL-22 (de Moura *et al* 2009)

It has been well established that IL-22RA2 is a naturally occurring antagonist of IL-22 (Xu *et al* 2001, Dumoutier *et al* 2001) and blockade of IL-22 signalling is achieved by binding the same binding site of IL-22 with higher affinity than IL-22RA1 (Diagram 3; de Moura *et al* 2009, Jones *et al* 2008, Wolk *et al* 2007, Wu *et al* 2008).

Expression

Immune system

Over the years, particular cells of the myeloid lineage (e.g. monocytes, macrophages, dendritc cells) have been shown to express IL-22RA2 mRNA. However, other immune cells such as tonsilar B cells (Xu *et al* 2001, Lecart *et al* 2002) and neutrophils (Whittington *et al* 2004) have been identified (Table 13).

Table 13. Expression of IL-22RA2 mRNA in cells of the immune system

Organ or Cell type	Detail	Evidence	Reference
PBMC	In Graft-vs-Host disease	IL-22RA2 mRNA	Yang *et al* 2007
Infiltrating mononuclear cells	Inflammation	IL-22RA2 mRNA	Xu *et al* 2001
Monocytes	Blood derived	IL-22RA2 mRNA	Whittington *et al* 2004
	Mouse monocytes	IL-22RA2 mRNA	Wei *et al* 2003
Macrophages	*In vitro* derived macrophages	IL-22RA2 mRNA	Wolk *et al* 2004
	Alveolar macrophages	IL-22RA2 mRNA	Whittington *et al* 2004
Dendritic cells	*In vitro* derived dendritic cells	IL-22RA2 mRNA	Wolk *et al* 2004
	Presumed dendritic cells (CD1a,CD34)	IL-22RA2 mRNA	Nagalakshmi *et al* 2004
	Presumed dendrtic cells (DC1)	IL-22RA2 mRNA	Nagalakshmi *et al* 2004
B cells	Activated CD19+ cells	IL-22RA2 mRNA	Xu *et al* 2001, Doumetier *et al* 2001
	Activated tonsilar B cells	IL-22RA2 mRNA	Xu *et al* 2001
Neutrophils	Alveolar neutrophils	IL-22RA2 mRNA	Whittington *et al* 2004

Structural cells

IL-22RA2 mRNA expression in structural cells is less well studied and, with a few exceptions, restricted to studies of tissue homogenates (Table 14).

Table 14. IL-22RA2 mRNA expression in the human tissue lysates

Organ/Region	Tissue/Cell type	Reference
Respiratory System	Lung	Xu *et al* 2001, Dumoutier *et al* 2001
	Type II pneumocytes	Xu *et al* 2001
Digestive System	Colon (weak)	Xu *et al* 2001
	Colon (strong)	Dumoutier *et al* 2001
	Human hepatoma cell (HepG2)	Wei *et al* 2003
	Small intestine (weak)	Xu *et al* 2001
	Pancreas	Xu *et al* 2001
Reproductive System	Placenta (very strong)	Xu *et al* 2001
	Breast (strong)	Dumoutier *et al* 2001
	Prostate (weak)	Xu *et al* 2001
	Testis	Dumoutier *et al* 2001
	Ovary (epithelial) carcinoma	Xu *et al* 2001
Integumentary System	Skin	Xu *et al* 2001
Cardiovascular System	Heart (weak)	Xu *et al* 2001
	Heart	Dumoutier *et al* 2001
Nervous System	Brain (weak)	Dumoutier *et al* 2001

Function of IL-22

General Function

The function of IL-22 has been studied in animal models in which IL-22 is either inhibited or administered in order to identify its functional significance in health and disease.

IL-22 administration

Intravenous injection of rIL-22 into mice was show to decrease the red blood cell count, serum albumin and body weight. Injection increased serum amyloid A1 (SAA) and fibrinogen

which led to basophilia in proximal renal tubules (Lambert *et al* 2001). The injection of IL-22 before inflammation was shown to inhibit hepatic cell damage (Radaeva *et al* 2004). While the administration of IL-17 changed the number of infiltrating neutrophils in the lung, IL-22 did not (Liang *et al* 2007). Adoptive transfer of IL-22- treated dendritic cells was observed to lower eosinophil counts in bronchoalveolar lavage fluid after ovalbumin challenge in ovalbumin (OVA)-sensitised mice (Schnyder *et al* 2010)

IL-22 blockade

Neutralising IL-22 before inflammation led to an increase in liver damage (Radaeva *et al* 2004). In the murine OVA-challenge model, IL-22 blockade increased eosinophil and neutrophil infiltration (Schnyder *et al* 2010). After IL-22 blockade in a Conconavalin A-induced injury model, IL-6 was lowered and IFNγ increased after Il-22 blockade (Wahl *et al* 2009). IL-22 was elevated in an IL-4R-/- knockout mouse and, when inhibited, led to an increase in eosiniphil recruitment, eosinophil peroxidase activity and slight increase in neutrophils (Schnyder *et al* 2010). IL-22 antagonism with rIL-22RA2 administration during infection showed reduced bacterial clearance and an increase in neutrophils, macrophages, CXCL1, TNFα, IL-6, and IL-10 (Weber *et al* 2007). In a murine model of colitis, blockade of IL-22 by IL-22RA2 administration suppressed goblet cell reconstitution during the recovery phase (Sugimoto *et al* 2008)

IL-22 Knock out mouse

It was shown that IL-22 deletion did not impact on the overall development of the mice. They were viable, fertile and had normal liver, spleen, pancreas, kidney, thymus and skin development (Zenewicz *et al* 2007). Injection of FASL into IL-22-/- mice showed no difference to the wild type suggesting that loss of IL-22 did not lead to increased apoptosis. They were fully susceptible to experimental autoimmune encephalitis (EAE) (Kreymborg *et al* 2007) and showed to mount a normal response towards *Listeria monocytogenes* (Zenewicz *et al* 2007). However, these mice were more sensitive to chronic inflammation (chronic hepatitis). This was later linked to an increase in the enzyme alanine transaminase (ALT) and increased hepatocyte damage which only occurred in the context of inflammation in IL-22-/- mice (Zenewicz *et al* 2008). This observation was unique and not seen in, for example, the IL-17-/- mouse. Investigation of IL-23-induced responses demonstrated that IL-22-/- mice showed lowered IL-23 induced ear skin ancanthosis and inflammation (Zheng *et al* 2007). This suggests that IL-22 may be a downstream mediator of IL-23 and contributor to acanthosis (epidermal hyperplasia). IL-22 -/- knockout mice also were shown to be more susceptible to oropharyngeal candidias infection (Conti *et al* 2009). During worm infestation

with *S.mansoni,* IL-22 -/- mice were shown to have increased levels of IL-17a and decreased levels of IL-4, IL-13, IL-10, and TNFα (Wilson *et al* 2010). IL-22-/- mice also demonstrated an association of IL-22 with BCL2 whose expression decreased by 20% compared to wildtype. It was also hypothesised that IL-22-/- mice have a breakdown of mucosal defences and increased risk of infection and inflammation. Cross of the IL-22-/- mice with the RAG2-/- mice resulted in a loss of IL-23 production. This suggested that innate IL-22 may be a trigger of IL-23 (Satoh-Takayama *et al* 2009).

Summary

An overview is given in the table below (Table 15).

Table 15. General functions of IL-22

Type	Evidence	Reference
IL-22 administration	Reduced Red blood cells[1], reduced Serum Albumin[1], reduced weight[1], SAA[1], fibrinogen[1], Basophilia in kidney[1], Protection of liver damage[2], IL-22 exposed DC caused less eosinophilia[3]	Lambert *et al* 2001[1], Radaeva *et al* 2004[2], Schnyder *et al* 2010[3]
IL-22 blockade	Increase in liver damage[1], Increase in eosinophils[2], increase in EPO activity[2], suppressed goblet cell reconstitution[6], reduced bacterial clearance[7]	Radaeva *et al* 2004[1], Schnyder *et al* 2010[2], Sugimoto *et al* 2008[6], Weber *et al* 2007[7]
IL-22 knockout	more sensitive to chronic hepatitis[1], increased liver damage[1], reduced ear skin ancanthosis and inflammation[2], mildly more susceptible to oropharyngeal candidias infection[3], decreased BCL2,-2/1 expression[4], Increased IgGc2 production[5]	Zenewicz *et al* 2008[1], Zheng *et al* 2007[2], Conti *et al* 2009[3], Satoh-Takayama *et al* 2009[5]

Cell specific functions

Immune cells

Immune cells are not generally believed to be a target of IL-22 but recent studies have identified that the addition of IL-22 reduced the CD80/86 expression on cultured dendritic cells (Schnyder *et al* 2010). In addition, macrophages have been shown to respond to IL-22 with increased phago-lysosomal fusion leading to improved killing of *Mycobacterium tuberculosis* (Dhiman *et al* 2009).

Structural cells

Epithelium

Epithelia are layers of cells covering internal or external surfaces. They share several important characteristics. They are tightly interconnected, show polarity (dipolar organisation & distribution), are attached to a thin basal lamina, are avascular and have a high rate of self renewal (through divisions of stem cells). They usually serve four essential functions. They control the passage of substances (physical barrier but also selective transport), control permeability, provide sensation and produce specialised secretions.

Keratinocytes

One of the general effects of IL-22 administration was increased proliferation of cells, thickening of the epidermis (Sa *et al* 2007) and the ability to improve the closure of an artificial gap or wound in a layer of cutaneous epithelial cells (Eyerich *et al* 2009). IL-22 was found to be strongly expressed in re-epithliasing skin wounds and IL-22 appeared to induce marapsin, a serine protease commonly associated with hyperproliferative epidermis (e.g. psoriasis) and regenerating wounds (Li *et al* 2009). IL-22 down regulated genes were found to be associated with cell differentiation and epidermal alterations (Nograles *et al* 2008). Several of the IL-22 induced immune genes in keratinocytes were also shown to be induced by IL-17a (e.g. CXCL1, CXCL8; Wolk *et al* 2009, CCL20; Harper et al 2009). More close investigation revealed synergistic effects between IL-22 and IL-17a on the upregulation of several immune response genes. These genes include HDB2, S100A7,-8,-9 (Liang *et al* 2006), CCL20, IL8, HB-EGF (Tohyama *et al* 2009) and IL-20 (Wolk *et al* 2009). Antagonisms between IL-22 and IL-17a have also been reported. These include the induction of GMCSF and VEGF by IL-17a (Koga *et al* 2008). Other interactions of IL-22 with other cytokines have been reported. TNFα and IL-22 together synergised in the induction of keratin-16 (K16) and CXCL8 (Wolk *et al* 2009). Interestingly, many genes, which were induced by synergies between TNFα and IL-22, were not observed in the IL-22 only treated cells. These included IL7, -15, -32, CCL2, -5, -20, -26, CXCL9, -10, -11, C1s, -r, CFH, CFB, TLR3, and -6 (Eyerich *et al* 2009). This would suggest that IL-22 boosts the action of the early inflammatory mediator TNFα and then, later on, to some extent the action of IL-17a. Some functional overlap was identified between IL-22 and IL-20. Both down regulated DSC1, K1,-10, CALM5 and LCE1B but together they did not synergise (Wolk *et al* 2009). Addition of rIL-22 to keratinocytes resulted in the up regulation of IL-1α, IL-6, TNFα, and the anti-microbial peptides S1008-9, DEFB1 and cathelicidin (Ma *et al* 2008). An overview is given in the table below (Table 16).

Table 16. Function of IL-22 in keratinocytes

Evidence	IL-22 administration	IL-22 blockade
General	Epidermal thickening[1] Proliferation independent of EGFR[1] Increased Wound closure[2,14] STAT1/3/5 phosphorylation[3]	
Protein	Increase IL-6[3], CXCL1[3], CXCL8[3], Marapsin[6], IL-20[10]	
mRNA (RT-PCR)	Increase S100A7[1,7,8,9,11,14], S100A8[9,14], S100A9[9,14], HDB2[9,14], CK16[1], TGFα[4], MIP3α[4], IL8[4,12], HB-EGF[4], CCL20[5], CXLC8[7,10], DEFB4[8], K16[10],MMP3[14],PDGFA[14], CXL5[14], Decreased: DSC1[10], K1[10], KRT10[10,14], CALM5[10], LCE1B[10]	
mRNA (Microarray)	Increase S100A7[1], S100A12[1], CCL20[1], CXCL14[1], DLX2[1], LCN2[1], C1s[1], VWP1[1], SNX10[1], HTR3A[1], SERPINA1[1], PRSS27[1], IL-1α[13], IL-6[13], TNFα[13], S100A8[13], S100A9[13], DEFB1[13], CAMP[13], Decreased: PPM1K[1], MSMB[1], APOL3[1], DCN[1]	

(source: Sa *et al* 2007[1], Eyerich *et al* 2009[2], Wolk *et al* 2004[3], Tohyama *et al* 2009[4], Harper *et al* 2009[5], Li *et al* 2009[6],Nograles *et al* 2008[7], Boniface *et al* 2007[8], Liang *et al* 2006[9],Wolk *et al* 2009[10], Pene *et al* 2008[11], Koga *et al* 2008[12], Ma *et al* 2008[13], Boniface *et al* 2005[14])

Airway Epithelial cells

Two recent studies have looked at the functional relevance of Il-22 (Aujla *et al* 2008, Sonnenberg *et al* 2010). Injured bronchial epithelial cells were shown to re-establish epithelial resistance more quickly when IL-22 was added. Synergies between IL-22 and IL-17 in IL-6 and CXCL1 up-regulation were confirmed and an increase in neutrophils ($Gr1^+$ cells) observed when both IL-22 and IL-17A were present in the lung (Sonnenberg *et al* 2010). In the transformed lung, alveolar epithelial cell line A549 IL-22 administration led to increased survival by up regulation of BCL2, and BCL-XL (Zhang *et al* 2008). The blockade of IL-22 led during stress led to a 20% decrease of BCL-2, BCL-2/1 expression (Sonnenberg *et al* 2010). This decrease was also observed when IL-17A was added to the IL-22 containing media, showing some antagonism between the two cytokines. An overview is given in the table below (Table 17).

Table 17. Function of IL-22 in lung epithelial cells

Evidence	IL-22 administration	IL-22 blockade
General	Re-establishment of barrier integrity GR1+cell increase[2]	
Protein	Increase G-CSF, IL-6[2]	Decrease IL-6, CXCL1, CCL3 (BAL fluid)
mRNA (RT-PCR)	Increase CSF, CSF3, LCN2, CXCL1[2], BCL2[3], BCL-XL[3]	Decrease BCL-2[2], BCL-2/1[2]
mRNA (Microarray)	Increase DEFB4, S100A7, S100A12, IL-19, IL-1F9, DUOX2, CXCL1, CXCL5, CCL3, CXCL9, MUC1, PIGR, SCGB3A1	

(source: Aujla *et al* 2008; Sonnenberg *et al* 2010[2], Zhang *et al* 2008[3])

Intestinal Epithelial cells

Similarly to keratinocytes, IL-22 restored the integrity of a physically injured layer of epithelial cells (Brand *et al* 2006, Pickert *et al* 2009). This function could be inhibited by PI3K inhibitors suggesting that migration played a major part in would closure while proliferation may only contribute to a minor extent (Brand *et al* 2006). The study of the IL-22-/- mice confirmed that the regulation of the two innate defence proteins REGIIIβ and –γ (Zheng *et al* 2008) was completely dependent on IL-22 while the protein expression for S100A8 and -9 only halved (de Luca *et al* 2010). A second study identified that TLR5 triggering by flagellin was one of the upstream initiators of IL-22 expression and downstream REG protein induction (Kinnebrew *et al* 2010). Studying the REG family of proteins more closely an IL-22-responsive element in the REGIa promoter region was identified (Sekikawa *et al* 2010). IL-22 helped the IFNγ induced expression of the two surface molecules of LY6A,-C which when crosslinked participate in chemokine release (Flanagan *et al* 2008). It was also identified that IL-22 was able to induce several mucin genes (MUC1, 3, 10, 13) and mucin secretion (Sugimoto *et al* 2008). IL-22 was shown to induce mainly Lipocalin2 (LCN2) while MUC4, CCL20 and NOS2 were also up regulated in a microarray analysis of IL-22 exposed T84 human colonic adenocarcinoma cells (Schulz *et al* 2008). An overview is given in the table below (Table 18).

Table 18: Function of IL-22 in intestinal epithelial cells

Evidence	IL-22 administration	IL-22 blockade
General	Wound closure by migration[1,4] Mucus production[8]	
Protein	Increase IL8[1],	Decrease: REGIIIβ[2], REGIIIγ[2], S100A8[2], S100A9[2]
mRNA (RT-PCR)	Increase IL8[1], hDB2[1], SOCS3[1], REGIa[3,7] REGIIIβ[5,7], REGIIIγ[5], LY6A[6], LY6B[6], MUC1[8], MUC3[8], MUC10[8], MUC12[8], LCN2[9]	
mRNA (Microarray)	Increase MUC4[9], CCL20[9], NOS2[9]	

(Brand *et al* 2006[1], de Luca *et al* 2010[2], Sekikawa *et al* 2010[3], Pickert *et al* 2009[4], Kinnebrew *et al* 2010[5], Flanagan *et al* 2008[6], Zheng *et al* 2008[7], Sugimoto *et al* 2008[8], Schulz *et al* 2008[9])

Endothelium

Blood-brain-barrier endothelial cells were shown to be IL-22RA1 (Kebir *et al* 2007) but the addition of IL-22 showed no alteration in the expression of the tight junction proteins occludin or Zo1.

Fibroblasts

So far, four different studies have identified fibroblasts as an IL-22 target cell population. IL-22 cultured subepithelial myofibroblasts were shown to have increased IL-19 and MMP1,-3 protein production (Andoh *et al* 2005). Synovial fibroblasts responded to IL-22 by increased cell growth and CCL2 protein production (Ikeuchi *et al* 2005). Skin fibroblasts responded to IL-22 with weak STAT3 phosphorylation (Wolk *et al* 2009). Human ganglia fibroblasts responded to IL-22 with CXCL10 protein release. This was either further increased by addition of IFNγ or decreased by TNFα (Hosokawa *et al* 2009). An overview is given in the table below (Table 19).

Table 19. Function of IL-22 in fibroblasts

Evidence	IL-22 administration	IL-22 blockade
General	Cell growth[2] STAT3 phosphorylation[3]	
Protein	Increase IL-19[1],MMP1[1], MMP3[1], CCL2[2], CXCL10[4]	
mRNA (RT-PCR)		
mRNA (Microarray)	Increase MMP1[1], MMP3[1], MMP10[1], CCL7[1], CXCL1[1], CXCL2[1], CXCL3[1], CXCL6[1], IL-6[1], IL8[1], IL-11[1], IL-13RA2[1], LIF[1], Follistatin[1], Stanniocalin1[1], SOCS2[1], Amphiregulin[1], Neuregulin1[1],	

(source: Andoh *et al* 2005[1], Ikeuchi *et al* 2005[2], Wolk *et al* 2009[3], Hosokawa *et al* 2009[4])

Specialised tissue cells

Hepatocytes

Hepatocytes are specialised cells which adjust several nutrient and mediator levels in the blood stream. In agreement with studies of epithelial cells, IL-22 facilitated reconstitution of a wounded layer of hepatocytes (Brand *et al* 2007). IL-22 showed to dampen aspartate aminotransferase (AST) and alanine transaminase (ALT) secretion (Yang *et al* 2010). IL-22 was shown to induce LPS-binding protein (LPSBP) to a similar level as seen with IL-6 (Wolk *et al* 2007. This induction was further enhanced in combination with IL-1β and TNFα. Blocking IL-22 with IL-22RA2 was shown to block all LPSBP suggesting that secretion of the protein was IL-22 dependent. Studying IL-22's role during viral infection of the liver (Hepatitis B and C) showed that it only slightly reduced viral burden (Dambacher *et al* 2008)

as well as demonstrated limited functional similarity to IFNλ but more functional similarity to IL-6 (Pagliaccetti *et al* 2010). In another study, administration of rIL-22 did not increase heptocyte proliferation but blockade of IL-22 led to a decrease in proliferation (Ren *et al* 2010). Blockade of IL-22 also led to a reduction in TNFα levels. An overview is given in the table below (Table 20).

Table 20. Function of IL-22 in hepatocytes

Evidence	IL-22 administration	IL-22 blockade
General	Wound closure[2]	Decreased proliferation[6]
Protein	Increase SAA[1], LPSBP[5] Decrease: AST[4], ALT[4]	Decrease: LPSBP[5], TNFα[6]
mRNA (RT-PCR)	Increase SAA[1], IL-6[3],IL8[3], TNFα[3], SOCS3[3]	
mRNA (Microarray)		

(Wolk *et al* 2005[1], Brand *et al* 2008[2], Brand *et al* 2007[3], Yang *et al* 2010[4], Wolk *et al* 2007[5], Ren *et al* 2010[6])

Regulation by IL-22RA2

While IL-22RA2 is known to bind IL-22 and block IL-22 signalling *in vitro*, very little is known about its occurrence and function *in vivo* (Table 21).

Table 21. Evidence of IL-22RA2 expression during inflammation

Model	Evidence	Function
Mouse model of Infection	mRNA up regulation 30 days post infection in the liver	Unknown (Wilson *et al* 2010)
Experimental Colitis model	Drop of IL-22RA2 mRNA expression in the inflamed intestine	Unknown (Wolk *et al* 2007)

Clinical associations of Il-22

Disease association

Allergy

With the identification of Th_{17} cells, allergic disorders were re-evaluated and shown to express their cytokines during phases of chronic and more severe phases of inflammation (Souwer *et al* 2010). The specific increase in IL-22 but not IL-17 was first identified during chronic atopic dermatitis supporting the occurrence of a novel Th_{22} T cell subset (Nograles *et al* 2009, Trifani *et al* 2010, Duhen *et al* 2009, Eyerich *et al* 2009). Apart from atopic dermatitis, a selective increase of IL-22 and IL-22RA1 expression was also identified in nickel contact dermatitis, a non-IgE mediated allergic disorder (Ricciardi *et al* 2009, Larsen *et al* 2008). Early studies in allergic asthma showed that IL-17$^+$ but especially IL-22$^+$ cells

were increased in T cells from peripheral blood while the number of IFNγ remained unchanged (Zhao *et al* 2009). Also in experimental asthma, using an OVA-challenge mouse model, IL-22 appeared to be increase more than IL-17 (Wang *et al* 2009).

Autoimmunity

Patients with systemic lupus erythmatosus (SLE) were shown to have decreased serum IL-22 expression which correlated with disease severity (Cheng *et al* 2009, Pan *et al* 2008). In the digestive system, IL-22 is associated with ulcerative colitis and Crohn's disease. Early on, high levels of IL-22 (especially from $CD4^+$Tcells) were identified in both conditions but the association of IL-22 was particularly significant in Crohn's disease (compared to ulcerative colitis; Andoh *et al* 2005). This high expression of IL-22 blood and tissue was confirmed (Wolk *et al* 2007) and correlated with disease severity (Schmechel *et al* 2008, Veny *et al* 2009 published online 2009). A mouse model for inflammatory bowel disease showed that IL-22 lead to reduction of colon thickness and overall disease score (Sugimoto *et al* 2008). In another model, blockade of IL-22 significantly delayed the recovery from colitis (Neufert *et al* 2010). An EAE mouse model showed that myelin oligodendrocyte antigen (MOG) led to a IL-22 (IL-17a, IFNg) increase (Kreymborg *et al* 2007, Thessen-Hedreul *et al* 2009). EAE still occurred when an IL-22 -/- knockout mouse was used (Kreymborg *et al* 2007).

However, the bulk of studies have researched the occurrence of IL-22 in psoriasis. Psoriasis is an autoimmune disease of the skin characterised by increased growth and proliferation of skin cells. Early on, a link between the cytokine and this skin condition was identified (Wolk *et al* 2005). It was shown that IL-22 was up regulated in infiltrating T cells and not in circulating T cells. Interestingly, the skin was shown to have reduced IL-22RA1 mRNA expression in these patients (Boniface *et al* 2007). Especially in psoriasis vulgaris, high IL-22 (and IFNg, IL-17a) was identified (Liu *et al* 2007, Lowes *et al* 2008). The relevance of IL-22 in the disease was confirmed when administration of anti-IL-22 blocked the disease development of murine psoriasis-like disease (Ma *et al* 2008). A genetic study showed that polymorphisms of the IL-22 in the population were not associated to chronic plaque psoriasis (Weger *et al* 2009). Another presumed autoimmune disorder, Vitiligo, which affects the skin, showed to be significantly associated with IL-22 expression. This was especially true when vitiligo was active (Ratsep *et al* 2008). High levels of serum IL-22 were found in autoimmune hepatitis (Dambacher *et al* 2008) and Hashimoto thyroiditis patients (Figueroa-Vega *et al* 2010). Patients with TNF-receptor-associated periodic syndrome (TRAPS) have a mutation in the TNFRSF1A gene which led to shedding of the TNFa receptor into the plasma thus

preventing normal TNFa signalling (Rezaei 2006) that was associated with increased levels of IL-22 (Nakamura *et al* 2010).

Autoimmune diseases associated with infection

Patients with chronic mucocutaneous candidiasis have reduced levels of IL-22 and IL-17a (Eyerich *et al* 2008) that correlated with the increased production of neutralising auto-antibodies against IL-22 (91%), IL-17F (75%) and IL-17a (41%) (Kiesand *et al* 2010, Puel *et al* 2010). Similarly, in autoimmune polyendocrine syndrome type I, auto-antibodies against IL-22 were identified (Puel *et al* 2010). In chronic sinusitis, decreased IL-22RA1 expression was associated with chronic disease and the development of nasal polyps (Ramanathan *et al* 2007) and it was later identified that polymorphisms in the IL-22RA1 gene highly correlated to disease severity (Endam *et al* 2009). An overview is given in the table below (Table 22).

Table 22. IL-22 association in autoimmunity

Disorder	Evidence	In	Reference
SLE	Decreased IL-22	Human	Cheng *et al* 2009
	Correlation to disease severity	Human	Pan *et al* 2009
Ulcerative colitis	High levels of IL-22	Human	Andoh *et al* 2005
Crohn's disease	Correlated to disease severity	Human	Schmechel *et al* 2008
	IL-22 lowered disease score	Mouse	Sugimoto *et al* 2008
	Delayed recovery when IL-22 is blocked	Mouse	Neufert *et al* 2010
			Kreymborg *et al* 2007
EAE	MOG antigen causes IL-22 expression	Mouse	Theussen-Hedreul *et al* 2009
	Not essential to disease development		Liu *et al* 2007
Psoriasis	High IL-22	Human	Ma *et al* 2008
	Blockade of IL-22 reduced disease severity	Mouse	Ratsep *et al* 2008
Vitiligo	Associated with IL-22 expression	Human	
Autoimmune hepatitis	High IL-22	Human	Dambacher *et al* 2008
Hashimoto thyroiditis	High IL-22	Human	Figueroa-Vega *et al* 2010
TNFRSF1A mutation	High IL-22	Human	Nakamura *et al* 2010
Chronic candida infection	Auto-antibodies against IL-22	Human	Kiesand *et al* 2010
			Puel *et al* 2010
Chronic sinusitis	Defect in IL-22RA1 gene	Human	Endam *et al* 2009

Infection

Viral

IL-22 production was increased during Hepatitis C infection (Dambacher *et al* 2008) and two single nucleotide polymorphisms (SNP) in the IL-22 gene were shown to affect the treatment response and viral clearance in patients (Hennig *et al* 2007). Yet, a mouse model of Heptatitis B infection showed that IL-22 indicated only limited antiviral properties and the response was different from the structurally related IFNλ (Pagliaccetti *et al* 2010). The IL-22

response was much more similar to that of IL-6. HIV-1 patients had high levels of IL-22 mRNA and protein expression while exposed individuals had less (Misse *et al* 2007). A link between IL-22 and the induction of the HIV1 protective SAA was postulated. According to the hypothesis, IL-22 induces SAA which down regulates of CCR5 expression on dendritic cells and, thus, limits viral spread. Chronic persistence of the Theiler virus in the central nervous system was linked to the 12q15 chromosome locus (Bureau *et al* 1993). While the locus harbours many genes including the two cytokines IL-22 and IL26, a mouse model of Theiler virus infection showed more direct evidence. In the model, IL-22 controlled mortality during acute encephalomyelitis (Levillayer *et al* 2007). In the serum of patients with the skin rash, pityriasis rosea, presumed to be triggered by herpes simplex, increased levels of IL-22 were found (Gangemi *et al* 2009). An overview is given in the table below (Table 23).

Table 23. Evidence of IL-22 association in viral infections

Viral infection	Evidence	In	Reference
Hepatitis B	IL-22 only limited antiviral activity	Human	Pagliaccetti *et al* 2010
Hepatitis C	Increased IL-22	Human	Dambacher *et al* 2008
	2 SNP in IL-22 gene affect positive treatment outcome	Human	Hennig *et al* 2007
HIV-1	High levels of IL-22 in infected patients	Human	Misse *et al* 2007
	IL-22 may induce SAA which lowers CCR5		
Theiler virus	More efficient immune response which also leads to increased mortality	Mouse	Levillayer *et al* 2007
Herpes simplex	Increased IL-22	Human	Gangemi *et al* 2009

Bacterial

Administration of Lipopolysaccaride (LPS), an endotoxin of gram-negative bacteria, led to the up regulation of IL-22 mRNA throughout the body (e.g. gut, thymus, spleen, lung, liver, kidney, stomach, heart; Dumoutier *et al* 2000). The gram negative bacteria Klebsiella pneumonia showed up regulation of IL-22 after 6 hours (Zheng *et al* 2008). Neutralisation led to the breakdown of tissue defences and systemic dissemination (Zheng *et al* 2008, Aujla *et al* 2008). This was only seen when IL-22 was blocked and not IL-17A (Aujla *et al* 2008). In a mouse model of mycobacterium tuberculosis (TB) infection, culturing macrophages with recombinant IL-22 improved their phagolysosomal fusion and their TB killing activity. In addition, it was shown that IL-22$^+$NK cells helped to reduce the overall TB burden (Dhiman *et al* 2009). In a primate model of TB infection, homing of IL-22$^+$ cells to the lung was observed at the late stage of infection (Yao *et al* 2010). A mouse model of Salmonella enterica infection showed that IL-22, together with IFNγ, was highly up regulated (Godinez *et al* 2008). In addition, protection was associated with IL-23 dependent IL-22 and not IL-17A (Schulz *et al* 2008). An overview is given in the table below (Table 24).

Table 24. Evidence of IL-22 association in bacterial infections

Bacterial infection	Evidence	In	Reference
LPS	Increase in IL-22	Mouse	Zheng *et al* 2008
Klebsiella pneumoniae	Increase in IL-22	Mouse	Zheng *et al* 2008
	Breakdown of tissue integrity when IL-22 blocked	Mouse	Aujla *et al* 2008
M. tuberculosis	Improved phago-lyso-somal killing of macrophages	Human	Dhiman *et al* 2009
Salmonella enterica	Increase in IL-22	Mouse	Godinez *et al* 2008
	Protection IL-23/IL-22 dependent not IL-17A	Mouse	Schulz *et al* 2008

Fungal

A mouse model of candida albicans infection showed that IL-22 was critical as a first line defence while IL-17A and IL-17F were dispensable (de Luca *et al* 2010). IL-22 was shown to contribute to epithelial integrity and control the yeasts growth. Interestingly, it was further up regulated in the absence of a Th_1 response. Blockade of IL-22 or IL-23 led to a decrease in T cell mediated defence against fungal hyphae (de Luca *et al* 2010). The saliva of IL-23p19 -/- knockout mouse exhibited lowered antifungal activity (Conti *et al* 2009).

Protozoan

Studying severe malaria anemia research identified that a polymorphism in the IL-22 (708^{th} position after ATG) was associated with protection which remained significant after stratification of the cohort according to ethnicity (European, African) (Koch *et al* 2005). Infection and induction of ileitis by the protozoan Toxoplasma gondii showed that IL-22 was associated with inflammation. In addition, IL-22, and not IL-17A, was IL-23 dependent and both cytokines were inversely regulated during the infection (Munoz *et al* 2009). Blockade of IL-22, therefore, displayed less intestinal pathology while the parasite burden remained unchanged. The decreased pathology was associated with overall reduced IL-17a, IL-17f, IFNγ and TNFα expression (Wilson *et al* 2010).

Other

Transplantation

In Graft versus Host disease, high levels of IL-22RA2 (together with IL2, IL2RA, AIRE) were found to be expressed in patients PBMCs (Yang *et al* 2007). A mouse model showed that transplants were rejected quicker in IL-22 deficient mice whereas in the wild type mice IL-22 production was identified to be in $Mac3^{+}CD11b^{+}$ cells and not lymphocytes (Kapessidou *et al* 2008).

Cancer

In two forms of cancer, ALK^+ anaplastic large cell lymphoma and non small lung carcinoma, expression of IL-22 and IL-22RA1 was shown to be linked to increased survival in mice (Zheng *et al* 2008, Bard *et al* 2008). Recently, a single nuclear polymorphism (rs1179251) in IL-22 was associated with colon cancer (Thompson *et al* 2010).

Arthritis

Arthritis can be caused by age and injury but can also have autoimmune triggers. In arthritic joints of patients, an increased number of $IL\text{-}22^+$ and $IL\text{-}17A^+$ T cell were found which were inversely related to T_{reg} cell numbers (Nistala *et al* 2008).

Asthma, COPD, ARDS, Pulmonary Sarcoidosis

Reduced levels of IL-22 were found in the epithelial lining fluid of ARDS patients (Whittington *et al* 2004). In asthma, early on, the 12q15 locus was identified in large scale genome association studies (Ober *et al* 1998, Satsangi *et al* 1996, Duerr *et al* 1998, Barnes *et al* 1996). While only two cytokine genes are found in this locus (IL-22, IL-26), the locus contains a further 66 genes (including 8 pseudogenes). Culturing T cells derived from peripheral blood ofasthmatic patients showed no alteration in IL-22 expression upon activaton (anti-CD3/CD28 and IL-23) compared to healthy individuals (Wong *et al* 2009). The phenotype of $CD4^+$ T cells from peripheral blood of asthmatics appeared to be mixture of IL-17A/IL-22 and IL-4 producing T cells (Cosmi *et al* 2010). In a mouse model for ventilation induced injury, IL-22 induced SOCS3 and reduced and lowered CXCL2, IL-6, and MMP9 and the overall biotrauma (Hoegl *et al* 2010). The causes of pulmonary sarcodis are unknown but it was shown that the bronchoalveolar lavage fluid of patients contained far less IL-22 protein as compared to that of healthy individuals (Whittington *et al* 2004).

Rationale

Allergy is an adverse immune reaction to an otherwise harmless environmental substance. The inflammatory response is characterised by IgE-dependent mast cell activation, eosinophil infiltration, and Th_2-cytokine release causing clinical symptoms such as itch (atopic dermatitis), runny nose and watery eyes (allergic rhinitis) or wheezing and shortness of breath (asthma).

While the predisposition to produce IgE and the inability to regulate the ensuing Th_2 response are a hallmark of the allergic response, non-immune contributions such as the role of a defective epithelium are increasingly recognised to play a role in disease progression. This is particularly evident, in asthma, where a potential reactivation of the EMTU may explain the observed aberrant tissue remodelling. Recent data also indicate a potential role of other T cell subsets in allergy and asthma. Whereas seasonal allergic rhinitis displays a classic Th_2-driven response (e.g. Th_2 cytokine expression and eosinophil activation; Durham 1992 & 1993), other atopic disorders are not as clearly defined. In atopic dermatitis, not only Th_2 cells increase during inflammation but also a delayed Th_1+Th_2 cell response can be observed (Yamanaka and Mizutani 2011). Similarly in asthma, the inflammatory response may not always be characterised Th_2 cytokine expression and eosinophilic infiltration (e.g. neutrophilic asthma). Similarly, the chronicity of inflammation may alter the normally Th_2 driven response leading to an increased T helper subset diversity.

IL-22 recently gained attention in chronic atopic dermatitis, where it was selectively increased by a new T helper cell subset, called Th_{22}. The cytokine IL-22 is structurally related to IL-10 and IFNλ. IL-22 signals through the IL-22RA1/IL-10RB signalling complex and is regulated by IL-22RA2, a soluble decoy receptor that binds IL-22 with high affinity. IL-22 is believed to be predominantly expressed by $CD4^+$ T cells while IL-22RA1 has been mainly identified on structural cells of epithelial origin. Many of IL-22's functions relate to the induction of pro-inflammatory mediators within epithelial cell, yet IL-22 has also been shown to regulate inflammation by dampening eosinophilic infiltration and contribute to tissue regeneration and repair.

While expressed in atopic dermatitis, the role of IL-22 in human allergy and asthma remains poorly understood. Outstanding questions therefore include: Is IL-22 expressed in human allergy and asthma? What is the dominant T cell subset expressing IL-22? Do Th_{22} cells represent a distinct T helper cell subset? What is the biological role of IL-22 in allergy and asthma?

Hypothesis, Aims, and Objectives

Hypothesis

Hypothesis 1:

Interleukin-22 is a T cell cytokine that is expressed during human allergic inflammation.

- This was tested by measuring Interleukin-22 and its receptors in bronchoalveolar lavage and skin biopsies after local allergen provocation

Hypothesis 2:

Interleukin-22 is produced by distinct Th_{22} cells as well as other T cell subsets (Th_1, Th_2, Th_{17}).

- This was tested by measuring Interleukin-22 production by peripheral-blood-mononuclear cells and T cells from peripheral blood, as well as T cell lines and clones derived from bronchial biopsies

Hypothesis 3:

Interleukin-22 is involved in tissue regeneration and repair.

- This was tested by evaluating the expression of Interleukin-22 receptors on human epithelial, smooth muscle and fibroblast lung cell lines and examining the influence of IL-22 on epithelial wound healing.

Aims and Objectives

In relation to Hypothesis 1: IL-22 is expressed in allergic inflammation

- Local segmental allergen challenge was performed in atopic asthmatics and controls. Bronchoalveolar Lavage was collected before and after allergen challenge and IL-22 concentrations were compared with levels of IFNγ, IL-5, and IL-17 as well as BAL eosinophil counts.
- Intradermal allergen challenge was performed in atopic allergic subjects. Cutaneous biopsies taken at 8 hours post challenge were analysed for IL-22 and its receptors. Results were compared with a control challenge using allergen diluent.

In relation to Hypothesis 2: IL-22 expression in human T cells

- Human peripheral-blood-mononuclear cells from grass pollen allergic subjects were cultured with Timothy-grass (*Phleum pratense*) allergen extract for 6 days. IL-22 expression was determined by ELISA and flow cytometry. Results for IL-22 and other cytokines were compared with PBMC [cultures] from non-atopic healthy controls.
- "Naive" ($CD4^+$ $CD45RA^+$) T helper cells were purified from PBMCs of grass pollen atopic donors. Expression of IL-22 and other cytokines after 2 weeks of culture in Th_{22}-polarising conditions (IL-6 + TNFα) was compared with IL-22 expression under Th_1-polarising conditions (IL-12, IFNγ, αIL-4).
- Freshly purified "memory" ($CD4^+$ $CD45RO^+$) T helper cells were obtained from PBMCs of normal healthy donors by negative selection. $CD4^+CD45RO^+$ cells were enriched for distinct chemokine receptors (CCR4, CCR6, CCR10) and putative novel Th_{22}-associated receptors (FGFR2, PDGFR, TIE1). The capacity of the enriched cells to produce IL-22 and Th_1/Th_2 and Th_{17} cytokines was examined by flow cytometry.
- T cell lines and clones obtained from bronchial biopsies of allergic and non-allergic asthmatics were examined by flow cytometry. IL-22 expression and co-expression with Th_1, Th_2, and Th_{17}-associated cytokines were examined by intracellular staining.

In relation to Hypothesis 3: IL-22 is involved in tissue regeneration and repair

- Structural cells from human lung were examined as potential target cells for IL-22. Cell lines from human lung epithelium, smooth muscle, and fibroblasts were examined for expression of IL-22 Receptors (IL-22RA1, IL-22RA2) by Western Blot and Flow cytometry. IL-22 Receptor regulation in response to stimulation by IL-22 and other T cell derived cytokines were analysed by RT-PCR and Flow cytometry.
- A human *in vitro* model of bronchial epithelial wound closure was developed to assess the potential function of IL-22 in regeneration and repair. Potential antagonism of IL-17 on IL-22 on wound healing was assessed in the same system
- Novel assays were developed to assess the mechanism of the effects of IL-22 on epithelial cell wound closure. These included quantifying epithelial cell proliferation and survival (reduction in apoptosis)

Materials and Methods

1. IL-22 expression in allergic inflammation

1.1. Local bronchial segmental allergen challenge

Participants

The study was approved by the ethics committee of the Royal Brompton and Harefield Hospital NHS Trust and written informed consent was given prior to the study. All study participants were selected using strict inclusion and exclusion criteria (Table 25). Regular use of inhaled gluocortico-steroids was discontinued 2 weeks before the start of the study and none of the participants required use of leukotreine receptor agonists. Note: For more detail on individual patient data please refer to appendix (Table 67, page **Error! Bookmark not defined.**).

Table 25. Inclusion and exclusion criteria for participants of the bronchial segmental allergen challenge

Inclusion Criteria	Exclusion criteria
Asthma (defined by clinical history, reversibility of airflow obstruction, and increased methacholine airway responsiveness) **Atopy** (against timothy grass pollen (*Phleum pratense*) or house dust mite (*Dermatophagoides pteronyssinus*), confirmed by positive skin prick test [≥5mm] and detectable allergen-specific IgE [RAST≥ IU/ml])	**Smoker** **Low Resting FEV_1** (less than 70% predicted) **Oral glucocortico-steroid use** (within 6 month prior to the study) **Allergen immunotherapy** (within 5 years prior to the study) **Neither** skin prick test positivity [≥5mm] **or** detectable allergen-specific IgE [RAST≥ IU/ml])

Segmental allergen challenge, Spirometry and Fibreoptic bronchoscopy

Participants were admitted to hospital for 24 hours. Normal values for forced expiratory volume (FEV_1) were predicted based on the participant's age, sex, height, weight and ethnicity (Quanjer *et al* 1993) and measured before and after allergen provocation. Broncho-alevolar lavage was taken immediately prior and 24 hours after allergen provocation. The peak expiratory flow rate was monitored hourly for the first 8 hours and then at 22 and 24 hours after challenge.

Bronchoalveolar lavage processing

The BAL was filtered through sterile gauze (50µm mesh), centrifuged at 1300rpm for 6 minutes and stored at -80°C for later use.

Eosinophil counts

The insoluble fraction of the BAL was stained for eosinophils using a May-Gruenwald-Giemsa stain (Fischer-Scientific ES8150). In brief:

The insoluble fraction of the BAL was re-suspended in Roswell Park Memorial Institute medium (RMPI 1640) and two drops applied to a microscope slide and smeared across using a cover slide. The slide was briefly air dried and placed for 30 seconds in Methanol. One drop of May-Gruenwald stain was then applied and left to stain for 5 minutes. The staining solution was then displaced using oxidising May-Gruenwald Wright-Giemsa buffer mixture and left for 3 minutes. The slides were then placed for 14 minutes in Giemsa Stain/Buffer mixture, then rinsed with Wright-Giemsa staining buffer for 2 minutes and left to air dry. The slides were then examined under light microscopy. Eosinophils were characterised based on a blue cytoplasm, blue to dark purple nuclei, and red to orange-red granules.

Cytokine ELISA

Commercially available ELISA assays were obtained from R&D Systems (Table 26) and used according to the manufacturer's instructions. In brief:

MaxiSorp 96-well plates (Nunc) were coated with 100µl per well (1/180 Phosphate buffered saline (PBS) diluted) capture antibody and left overnight at room temperature. Plates were washed 3 times with wash buffer (0.05% Tween-20 [Sigma P1379] PBS) and blocked for 1 hour using filtered 1% BSA PBS. Plates were washed 3 times and cytokine standards and samples were added for 2 hours. Plates were washed again 3 times and 100µl of (1/180 1% Bovine serum albumin (BSA) PBS-diluted) detection antibody was added and left to bind for 2 hours. Plates were washed 3 times and 100µl of (1/10 in 1%BSA PBS-diluted) Streptavidin- Horseradish peroxidase (HRP) was added per well for 20 minutes. Plates were washed again 3 times and 100µl of 5,5-Tetramethylbenzidine (TMB) substrate solution was added and left in the dark for 20 minutes. The reaction was stopped using 100µl of 1.8M sulphuric acid and analysed immediately using a Teacan Sunrise optical plate reader. Optical density readings were obtained at 450nm and 540nm (for background correction) and a standard curve was plotted. The equation of the line was determined for standards within linear range and used to calculate the concentrations of unknown samples.

Table 26. Cytokine ELISA assays utilised throughout the study

Name	Company	ID	Detection Limit
Human IFNγ Duoset	R&D Systems	DY285	15pg/ml
Human IL-5 Duoset	R&D Systems	DY205	8-15pg/ml
Human IL-17 Duoset	R&D Systems	DY317	15pg/ml
Human IL-22 Duoset	R&D Systems	DY782	15pg/ml

IL-22RA2 ELISA

The IL-22RA2 Sandwich ELISA was developed using two anti-IL-22 antibodies binding different IL-22 target epitopes. The only available antibody combination consisted of 2 polyclonal antibodies raised both in goat (Table 27).

Table 27. Antibodies incorporated in the IL-22RA2 sandwich ELISA design

Name	Company	ID	Epitope	Stock Concentration
IL-22RA2 (T16)	Santacruz biotechnology	sc-67640	Internal region	200µg/ml
IL-22RA2 (N16)	Santacruz biotechnology	sc-67637	Near N-terminus	200µg/ml
IL-22RA2 (T16)-BIOTIN	*created during PhD*	*Not available*	Internal region	1mg/ml

Validation of IL-22RA2 antibodies

Maxisorp ELISA plates (NUNC) were coated overnight with 100µl of PBS containing either different concentrations of recombinant IL-22RA2-Fc chimera (R&D) or protease free bovine serum albumin (PAA K45-012). The ELISA was carried out as previously described. For detection a horse anti-goat-biotin-labelled secondary antibody (Vector BA-9500) diluted in 1%BSA PBS was incorporated.

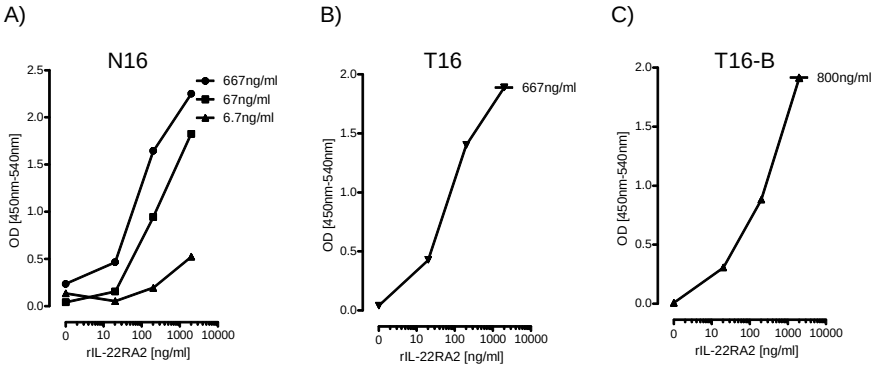


Figure 7. Validation of α-human IL-22RA2 antibody (N16, T16, T16-biotin) binding by direct ELISA using plate bound recombinant L-22RA2.

A) N16 antibody B) T16 antibody C) T16-Biotin labelled antibody

The results showed that increasing concentrations of IL-22RA2 antibodies led to a liner increase in optical density (OD) (Figure 7A-B).

Biotin labelling

To label the IL-22RA2 (T16) antibody with biotin its concentration had to be increased from 200µg/ml to 1mg/ml. To do so 30kDa cut off spin filter tubes (Millipore Amicon) were employed. The centrifugation was carried out at 2500rpm and a total volume of 500µl was reduced to 100µl giving a concentration of 1mg/ml. The Biotin labelling was carried out using the Biotin-XX Microscale Protein labelling kit (Invitrogen B30010). In brief:

100µl of 1mg/ml IL-22RA2 (T16) antibody was transferred to a supplied reaction tube. 10µl of 1M sodium bicarbonate was added into the tube and mixed by pipetting. 10µl of deionised water was added to one vial of reactive biotin. The amount of required reactive biotin was calculated using following formula:

$$\frac{\mu g\ of\ protein}{MW\ of\ protein}\ x\ 1000x\ Molar\ Ratio \Big/ _{14.39} = \mu l\ of\ reactive\ biotin$$

In this case 100µl of 1mg/ml IL-22RA2 antibody (MW= 144kDa) and a molar ratio of 18 (see manufacturer guidelines) required 0.84µl reactive biotin.

$$\frac{100\ \mu g}{144000\ Da}\ x\ 1000x\ 18 \Big/ _{14.39} = 0.84\ \mu l$$

This amount was transferred into the reaction tube and mixed by pipetting and left to react at room temperature for 15 minutes. The reaction solution of 100µl was split between 2 previously prepared and activated gel resin spin filter tubes. The two spin filter tubes were then centrifuged at 16000g for 1 minute. The biotin-labelled antibody was then validated by direct ELISA (Figure 7C, page 72).

IL-22RA2 Sandwich ELISA

The sandwich ELISA was carried out as previously described using the IL-22RA2 (N16) antibody as capture and the IL-22RA2-Bition antibody for detection.

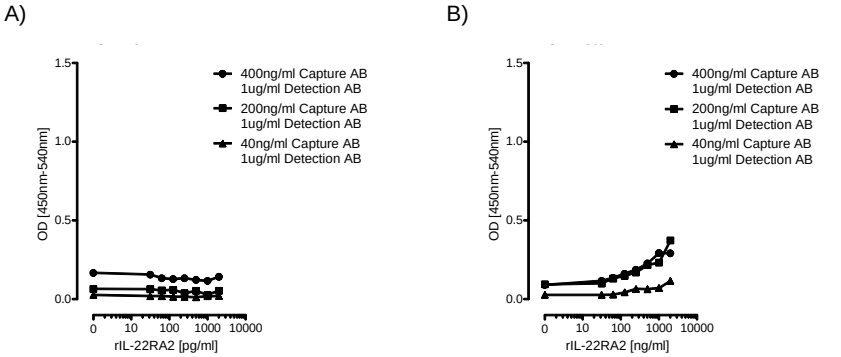


Figure 8. Detection of fluid phase rIL-22RA2 in pg/ml and ng/ml range using a the sandwich IL-22RA2 ELISA protocol

A) pg/ml rIL-22RA2 range B) ng/ml rIL-22RA2 range

The results showed that without optimisation, the sandwich ELISA produced a weak non-linear signal in ng/ml range but was unable to detect IL-22RA2 in pg/ml range (Figure 8A-B).

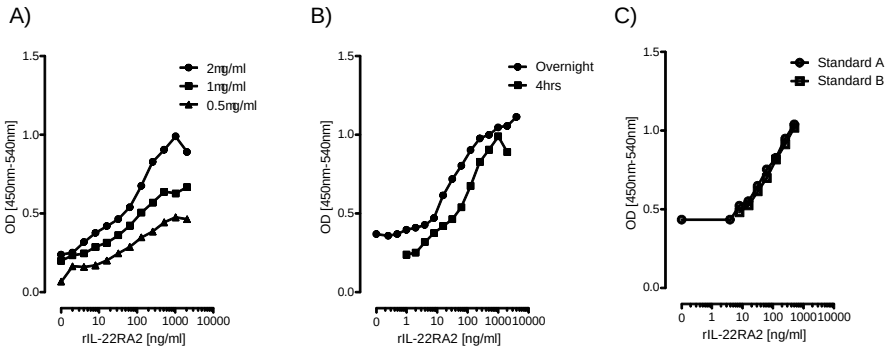


Figure 9. Optimisation of antibody concentrations and detection time within the sandwich IL-22RA2 ELISA protocol

A) Detection antibody concentrations B) Detection time C) Final ELISA protocol

Changes in antibody concentrations, detection time, streptavidin-peroxidase ratios (Figure 9A-B) led to an improved ELISA protocol (Table 28) enabling detection of increasing rIL-22-RA2-Fc concentrations of 8-500ng/ml in a liner fashion (Figure 9C).

Table 28. Final IL-22RA2 Sandwich ELISA protocol

ELISA steps	Reagent	Company	Concentration	Incubation time
Capture antibody	αhu IL-22RA2	SCBT (sc67640)	1:500 (400ng/ml)	Overnight
Detection Antibody	αhu IL-22RA2-biotin	*Based on SCBT (sc67637)*	1:1000 (1µg/ml)	4 hrs
Streptavidin-Enzyme	Extravidin-Peroxidase	Sigma (E2886)	1:1000	45 min
Enzyme substrate	TMB liquid solution	Sigma (T0440)	neat	30 min (max)

1.2. Intradermal allergen challenge

Participants

The study was approved by the ethics committee of the Brompton and Harefield Hospitals NHS Trust and informed written consent was obtained prior to the study. Participants were classified using their clinical history, current allergen skin prick responses and allergen specific serum IgE concentrations. The inclusion and exclusion criteria are listed in table 29. Note: For more details on individual patients please refer to appendix (Table 68, page **Error! Bookmark not defined.**).

Table 29. Inclusion and exclusion criteria for participants of the intradermal allergen challenge study

Inclusion	Exclusion
Age 18-55 years	**Atopic group**
Atopic group	History of serious allergic responses incl. anaphylaxis
Seasonal allergic rhinoconjunctivitis for at least 2 years	History of immunotherapy within last 5 years
Positive skin prick response (>3mm) and RAST levels greater > IU/ml	No use of anti histamine 10 days prior to the study
	Severe atopic dermatitis or asthma requiring medication
Normal control group	**Both groups**
No clinical history of allergy, no skin prick response, no RAST	The use of prednisolone or other immunosuppressive agents
	Pregnant or breastfeeding
	Evidence of alcohol or drug abuse
	Any significant systemic disease

Intradermal allergen challenge, Late phase response and Punch biopsies

The intradermal challenge was performed by medical staff at the Brompton hospital outside the UK pollen season. 10 biological units of timothy grass extract in 0.02ml (ALK Abello, Denmark) were given to the extensor surface of one forearm. An injection of allergen diluents was administered to the opposite forearm. After 8 hours, the size of the response was measured. Under local anaesthetic (2% Lidocaine), a 4mm biopsy was removed from the challenge site using a sterile punch, forceps and scalpel. The wound was then closed using two interrupted 5.0 Nylon sutures.

Biopsy processing

Cut into half, one part of the biopsy was placed into 4% paraformaldehyde containing PBS for 2 hours and then transferred into 15% sucrose PBS for 1 hour. Both treated and untreated halves were embedded in drops of optimal cutting temperature (OCT) medium (Tissuetek, England), placed on labelled cards and dipped into liquid-nitrogen cooled

isopentane solution (BDH 103614T). From these 5 µm thin tissue sections were cut using a Bright OTF Cryostat (Bright Instrument Company, UK) and placed on Superfrost Plus Gold microscope slides (BDH chemicals, UK). Slides destined for acetone fixation were left for 10 minutes in ice-cold acetone and then briefly air dried. Acetone and previously paraformaldehyde fixed tissue slides were then wrapped in aluminium foil and stored at -80°C.

Eosinophil counts

Eosinophils were detected using a mouse anti-human Major Basic Protein antibody (clone BMK-13, donated by Barry Kay & Julie Barkans) at 1/30 dilution and detected using the Vector anti mouse horseradish-peroxidase detection kit. The eosinophils were detected and quantified by light microscopy.

Immunofluorescent staining

General fluorescent staining protocol

Slides were defrosted at room temperature and each tissue section was encircled using water resistant ImmEdge pen (Vector Laboratories H-4000). Slides were pre-treated for 30 minutes in 0.1% saponin containing PBS. Slides were blocked using 20% donkey serum (Jackson 017-000-01) PBS for 30 minutes. The serum was tipped off and the primary antibodies diluted in 5% normal human serum PBS were added for 1 hour. Slides were washed 3 times for 5 minutes in 1X PBS before the second layer of antibodies (e.g. donkey anti-goat-biotin) diluted in 10% normal human serum PBS were added for an additional 1 hour. The wash steps were repeated and the third layer was added (e.g. streptavidin-Alexa Fluor 594) for 30 minutes in the dark. (Note: Streptavidin-Alexa conjugates were centrifuged at 13000rpm for 3min before use). Slides were washed again and 1 drop of Vectashield containing 4',6-diamidino-2-phenylindole (DAPI) was added and the slide and covered with a cover slip. Slides were analysed using a Nikon Eclipse E400 Fluorescent Microscope. Digital images were obtained and further analysed using Lucia 4.8 software. Average cell counts were derived from >10 images obtained through a checker board system and converted from cells per section to cells per mm^2 (Table 30). Note: Please refer to table 31 for complete list of antibodies used.

Table 30. Conversion factors for histological cell counts

Magnification	Dimension of the image (mm)	Area of the image (mm^2)	Multiplication factor (cells/mm^2)
100x	1.19 x 0.940	1.1186	0.9
200x	0.60 x 0.474	0.2844	3.5
400x	0.30 x 0.237	0.0711	14

Table 31. Antibodies and reagents used in the immunofluoresent analysis of allergen challenged skin

Name	Species	Company	ID	Concentration used
α-IL-22	Goat polyclonal	ABcam	AB18498	Various (20µg/ml)
α-IL-22	Goat polyclonal	Santa Cruz Biotechnology	Sc-14437	Various (20µg/ml)
α-IL-22	Goat polyclonal	R&D Systems	AF782	Various (20µg/ml)
α-IL-22RA1	Goat polyclonal	Santa Cruz Biotechnology	Sc-67640	1/100 (2µg/ml)
α-IL-22RA2	Goat polyclonal	Santa Cruz Biotechnology	Sc-67637	1/100 (2µg/ml)
α-CD3	Mouse monoclonal	Dako	M7254	1/15
α-CD3	Rabbit polyclonal	Abcam	ab28364	1/10 (200µg/ml)
α-CD31	Mouse monoclonal	Dako	M0823	1/40
α-CD31	Rabbit polyclonal	Abcam	ab28364	1/100
α-Vimentin	Mouse monoclonal	Dako	M7020	1/100 (0.27µg/ml)
α-Vimentin	Goat polyclonal	Abcam	ab89996	1/200 (5µg/ml)
α-CD68	Mouse monoclonal	Dako	M0718	1/26.5 (20µg/ml)
α-Neutrophil elastase	Mouse monoclonal	Dako	M0752	1/100 (1.1µg/ml)
α-Mast cell tryptase	Mouse monoclonal	Dako	M7052	1/42.5 (2µg/ml)
α-Major basic protein	Mouse monoclonal	AbD Serotec	4180-2008	1/100 (2µg/ml)
α-HSP47	Mouse monoclonal	Enzo Life Sciences	ADI-SPA-470-D	1/800 (1.25µg/ml)
α-alpha SMA	Mouse monoclonal	Dako	M0851	1/800 (87.5pg/ml)
Goat isotype	Goat polyclonal	R&D Systems	AB-108-C	Various
Mouse isotype	Mouse monoclonal	Santa Cruz Biotechnology	sc-3877	Various
Rabbit isotype	Rabbit polyclonal	Abcam	ab125938	various
α-goat IgG-BIOTIN	Donkey polyclonal	Jackson	705-065-003	1/170
α-mouse IgG-BIOTIN	Goat polyclonal	Vector	BA-9200	1/300 (5µg/ml)
α-rat IgG-BIOTIN	Goat polyclonal	Vector	BA-9400	1/300 (5µg/ml)
α-rabbit IgG-TRITC	Swine polyclonal	Dako	R0156	1/200 (7.5µg/ml)
α-mouse IgG-FITC	Rabbit polyclonal	Dako	F0261	1/30 (13.3µg/ml)
α-goat IgG-FITC	Rabbit polyclonal	Dako	F0250	1/30 (1µg/ml)
Streptavidin-A488	-	Molecular Probes	S11223	1/1250 (1.6µg/ml)
Streptavidin-A594	-	Molecular Probes	S11227	1/400 (5µg/ml)

Validation of IL-22 antibodies

Memory $CD4^+$ T cells were obtained from peripheral blood and polyclonally activated as previously described. Using a Shendon-3 cytospin centrifuge, 100µl of $0.3x10^6$ cells/ml cell suspension were added to each cytospin holder. Each holder contained filter paper, microscope slide and cytospin funnel and was centrifuged for 3 minutes at 450rpm. The

slides were air dried, fixed for 15 minutes in 4% paraformaldehyde (BDH 294474L) PBS and transferred into 15% sucrose (BDH 102747E) PBS for 15 minutes. The slides were then transferred to a second sucrose solution for 15min before being rinsed in PBS and dried overnight using an oven (at 37°C). The slides were then checked by light microscopy for successful cell adherence and intact cell morphology by staining two randomly selected slides with 2 drops of Harris haematoxylin solution (VWR 351945S). Successful cytospins were wrapped in aluminium foil and stored at -80°C.

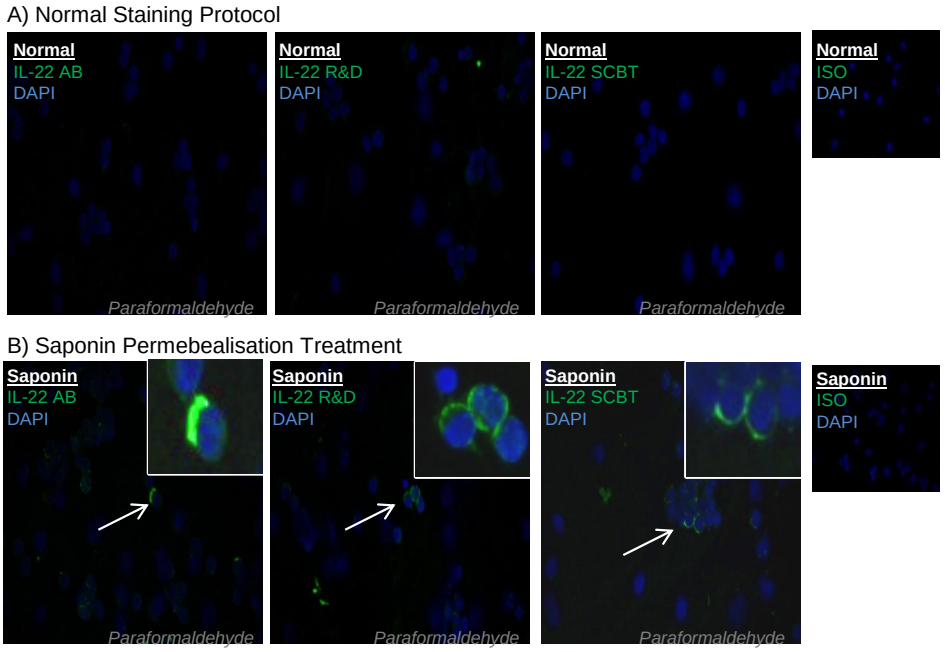


Figure 10. IL-22 Immunofluorescent staining of cytospin preparations of polyclonally stimulated (PMA/Ionomycin) and paraformaldehyde-fixed peripheral blood $CD4^+$ $CD45RO^+$ T cells

A) Regular Immunofluorescence staining protocol B) pre-treatment of slides with Saponin.

IL-22 antibodies were validated using paraformadehyde-fixed cytospin preparations of activated peripheral blood $CD4^+CD45RA^-$ T cells (Figure 10). The results showed that a small proportion of memory T cells stained positive using any of the three anti-human IL-22 antibodies after permebelisation (saponin treatment).

2. IL-22 expression in T cells

2.1. from peripheral blood

2.1.1. long term allergen PBMC culture

Participants

Study participants were recruited by the medical staff at the Brompton hospital (London, UK) based on their clinical history of allergic rhinitis, positive timothy grass pollen skin prick test, and significantly elevated timothy grass specific serum IgE levels. Recruited normal healthy control participants had no reported history of allergy. In addition normal control participants tested negative for common allergens by skin prick testing, had no elevated levels of allergen specific IgE or total IgE. Prior to the study all participants had given written informed consent. For more details on individual patients please refer to appendix (Table 69, page **Error! Bookmark not defined.**).

PBMCs from peripheral blood

Blood was taken by medical staff at the Brompton hospital and transferred within 30 minutes to the laboratory for PBMC isolation by Ficoll-Paque separation. In brief:

The blood was diluted 1:4 using sterile PBS and 30ml was layered on top of 15ml of Ficoll-Paque Plus (GE 17-1440-03) in 50ml Falcon tubes and then centrifuged at 2000 rpm for 25 minutes (without brakes) inside swing-buckets (Hettich Rotina 420R centrifuge). After centrifugation the PBMC layer was taken off using a sterile plastic dropper and collected in a new 50ml falcon tube. The dense PBMC solution was diluted using PBS and centrifuged at 1500rpm for 10minutes. The PBS was discarded and the cell pellet suspended in fresh PBS and centrifuged once more at 1500rpm for 10min. After the final wash, the cell pellet suspended in a small volume of PBS and counted using a haemocytometer.

In vitro culture

PBMCs were cultured for 6 days in RPMI-1640 +L-Glutamine (Invitrogen/Gibco 52400) substituted with 5% human AB Serum and 1/10 diluted Penicilin/Streptomycin Solution (Invitrogen 15140-122). Depending on the condition the tissue culture media contained either no stimulus, 5µg/ml whole *Phleum pratense* extract (ALK-Abello 11372), 10µg/ml PHA (Sigma-Aldrich L1668), 10µg/ml Tuberculin purified protein derivative (PPD) (Statens Serum Institute 2390).

[methyl^{3}H]-Thymidine incorporation

Using a 96-well U-bottomed plate (Nunc 167008) 5x10^5 PBMCs were added to each 96- well in a total volume of 200µl tissue culture media and incubated at 37°C with 5%CO_2. After 6 days, 20 µl of 1/20 RPMI-diluted [Methyl-$H^{3]}$-Thymidine solution (5mCi/185 MBq; MP Bio 2405905) was added to each well. After 16hrs at 37°C the cells were harvested using a 96-well plate harvester (Tomtec Harvester 96 Mach III-M). Before harvesting, the harvester was washed twice using the wash-aspirate program and an empty 96 well plate. Then a filter mat (PerkinElmer 1450-421) was added and pulse-washed. The empty plate was exchanged with the sample plate and the cells were pulsed onto a filter mat using the pulse-wash program. The filter mat was removed from the harvester and left to dry for 1 hour at room temperature. After air drying, the filter mat was inserted into a small plastic bag (PerkinElmer 1450-432) together with 4 ml of Scintillation fluid (PerkinElmer 1205-440) and sealed using an electric heat sealing machine (Hulme Martin). The scintillation fluid was distributed evenly across the filter mat using a roller. Radioactivity was measured using a Scintillation counter (Wallac Trilux 1450 Microbeta counter) and background corrected.

Cytokine ELISA

100µl of PBMC culture supernatant was analysed as previously described (see Method 1.1, page 71)

Flow Cytometry

Activation

Cells were washed twice in fresh culture media and re-suspended at 1x10^6 cells/ml. Using a 24-well plate (NUNC) cells were polyclonally activated for 6 hours using 20ng/ml phorbol myristate acetate (PMA) and 1µg/ml ionomycin. In addition 0.6µl/ml GolgiStop was added from the start and 1µl/ml GolgiPlug after 2 hours of culture to stop protein secretion.

Staining and fixation

200µl containing 2x10^5 cells were transferred per well into a 96-well plate. The plate was centrifuged at 1500rpm for 5 minutes and the media removed. 20µl of Fc blocking solution (1/10 diluted Mitenyi Fc block stock solution) was added to per well and left in the dark for 20 minutes at room temperature. Surface FACS antibodies (e.g. CD4 and CD8) and their respective isotype controls were added into each well at a ratio of 1/20 and left for 30 minutes in the dark at 4°C. The plate was washed using 250µl Perm/Wash buffer per well and centrifuged at 1500rpm for 5 minutes. The Perm/Wash buffer was replaced with 100µl of BD Cytofix/Cytoperm solution and left for 20 minutes to incubate in the dark at 4°C. The plates were washed twice with 250µl Perm/Wash buffer and the intracellular FACS

antibodies were added at a ratio of 1/20 into final volume of 20µl of Perm/Wash buffer. The cells were left in the dark for 30 minutes at 4°C and then washed twice more using 250µl Perm/Wash buffer. The cells were then fixed in 200µl BD Cellfix solution (BD 340181) and stored at 4°C overnight. Individual FACS antibodies used are given in the table below (Table 32).

Table 32. FACS antibodies used in the analysis of T cells derived from allergen PBMC co-cultures

Name	Species	Company	Clone	ID	Concentration used
α-CD4-PE CY7	Mouse IgG1	BD Bioscience	SK3	557852	1 in 20
α-CD8-Amcyan	Mouse IgG1	BD Bioscience	SK1	339188	1 in 20
α-IFNγ-V450	Mouse IgG1	BD Bioscience	B27	560371	1 in 20
α-IL-4-A488	Mouse IgG1	BD Bioscience	8D4-8	557990	1 in 20
α-IL-5-PE	Rat IgG2a	BD Bioscience	JES1-39D10	554489	1 in 20
α-IL-9-PERCP CY5.5	Mouse IgG1	BD Bioscience	MH9A3	561461	1 in 20
α-IL-17A-A488	Mouse IgG1	R&D Systems	41802	IC3171A	1 in 20
α-IL-22-PE	Mouse IgG1	R&D Systems	142928	IC7821P	1 in 20
α-IL-22-APC	Mouse IgG1	R&D Systems	142928	IC7821A	1 in 20
Isotype-PE CY7	Mouse IgG1	BD Bioscience	MOPC-21	557872	1 in 20
Isotype-Amcyan	Mouse IgG1	BD Bioscience	X40	339185	1 in 20
Isotype-V450	Mouse IgG1	BD Bioscience	X40	642268	1 in 20
Isotype-A488	Mouse IgG1	R&D Systems	G94-56	554008	1 in 20
Isotype-PE	Rat IgG2a	BD Bioscience	R35-95	554689	1 in 20
Isotype-PERCP CY5.5	Mouse IgG1	BD Bioscience	MOPC-21	550795	1 in 20

Acquisition and analysis

The fixed cells were assayed using a FACS Canto II machine. Lymphocytes were pre-selected using the forward and side scatter. Fluor chrome compensation was carried out using the auto compensation setup of the FACS Canto machine. The cytometer settings were saved and used for all acquisitions. In total, 10000 cells were acquired and analysed using the BD FACS DIVA software.

2.1.2. polarised naive T cells

T cell isolation from PBMCs

PBMCs from healthy non-atopic donors were obtained by Ficoll-Paque isolation as previously described (see Methods 2.1.1, page 79). $CD4^+$ T helper cells were isolated from PBMCs by depleting non-$CD4^+$ T cells such as $CD8^+$ $CD14^+$ $CD16^+$ $CD19^+$ $CD36^+$ $CD56^+$ $CD123^+$ $TCR\gamma/\delta^+$ Glycophorin A^+ cells. The obtained fraction was further depleted using CD45RO and enriched using CD45RA beads to obtain $CD4^{(+)}$ $CD45RO^-$ $CD45RA^+$ cells (Table 33).

Table 33. Magnetic bead isolation kits used in the enrichment of individual T cell populations

Isolation kit	Type	Company	ID
$CD4^+$ T Cell Isolation Kit II	negative selection	Miltenyi Biotec	130-091-155
CD45RA Microbeads	positive selection	Miltenyi Biotec	130-045-901
CD45RO Microbeads	positive selection	Miltenyi Biotec	130-046-001

Magnetic labelling

The PBMC cell pellet was re-suspended in 40µl MACS buffer (0.5% BSA 2mM EDTA PBS) per 10^7 cells. 10µl of non-CD4 depletion antibody cocktail was added per 10^7 cells, mixed, and incubated for 10 minutes at 4°C. 30µl MACS buffer and 20µl anti-biotin magnetic beads were then added in succession, mixed and left for 15 minutes at 4°C. Cells were then washed using 10x volume of MACS buffer and centrifuged at 1500rpm for 10 minutes. The supernatant was taken and the cell pellet was re-suspended in 500µl MACS buffer per 10^8 cells.

Magnetic isolation

A large sized (LS) magnetic column (Miltenyi) was used to isolate a maximum of 10^8 cells from a given cell suspension. The column was placed into a supplied magnet and 3ml 1%BSA PBS was rinsed through before the cell suspension was applied. The unlabelled cells were collected and the LS column washed 3x using 3ml 1% BSA PBS. The magnetic field was removed and the labelled cells eluted using 3ml of 1% BSA PBS. Both labelled and unlabelled fractions were counted using a haemocytometer and either used directly or labelled for additional magnetic bead isolation (e.g. CD45RO depletion).

Validation of naive T cell isolation

The isolated "naïve T cell"-fractions were analysed by FACS as previously described (see Methods 2.1.1, page 80) using FACS antibodies listed in the table below (Table 34).

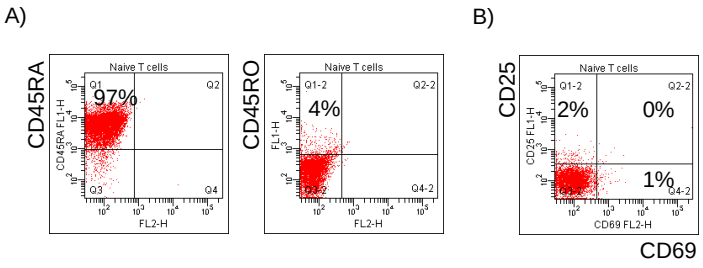


Figure 11. Flow cytometric analysis of CD45RA,-RO, CD25, and CD69 expression on purified naive peripheral blood T cells used in CD4⁺ T cell differentiation

Surface expression of A)CD45RA and CD45RO as well as B) T cell activation marker CD25 and CD69

The results showed that CD45RA expression was >95% and CD45RO expression less then <5% (Figure 11A) and both activation markers CD25 and CD69 expression were low (<2%) or absent (Figure 11B).

Table 34. FACS antibodies used in validating T cells after magnetic bead isolation

Name	Species	Company	Clone	ID	Concentration used
α-CD45RA-FITC	Mouse IgG1	BD Bioscience	OX-33	554883	1/20
α-CD45RO-FITC	Mouse IgG2a	BD Bioscience	UCHL1	555492	1/20
α-CD25-FITC	Mouse IgG1	BD Bioscience	OX-39	554865	1/20
α-CD69-PE	Mouse IgG1	BD Bioscience	L78	341652	1/20
Isotype-FITC	Mouse IgG1	R&D Systems	11711	IC002F	1/20
Isotype-FITC	Mouse IgG2b	R&D Systems	133303	IC0041F	1/20
Isotype-PE	Mouse IgG1	BD Bioscience	MOPC-21	551436	1/20

T helper cell differentiation

The original Th_{22} *in vitro* differentiation protocol (Duhen *et al* 2009):

Naïve T cells were isolated from buffy coats by flow assisted cell sorting as $CD4^+$ $CD45RA^+$ $CCR7^+$ $CD45RO^-$ $CD25^-$ cells to a purity of 99%. Naïve T cells ($5x10^4$) were cultured in U-bottom 96 well plates in the presence or absence of 10ng/ml IL-6 and 5ng/ml TNF. Cells were stimulated using beads coated with anti-CD3 and anti-CD28 at a ratio of 1 cell per 1 bead. After 5 days, cultures were supplemented 50U/ml IL-2. At day 12, cells were activated using PMA and Ionomycin for 5 hours, stained for intracellular cytokines and assessed by flow cytometry.

Alterations made:

Fresh blood was used instead of buffy coat and naive T cells were obtained through magnetic bead isolation rather than FACS isolation. Both serum-free (AIM-V, Invitrogen/Gibco 12055-091) and serum-containing RPMI-1640 +L-Glutamine (Invitrogen/Gibco 52400) substituted with 5% human AB Serum and 1/10 diluted Penicilin/Streptomycin Solution (Invitrogen 15140-122) media were trialled. In addition we used a range of cytokine concentrations and carried out Th_{22} priming in the presence and absence of anti-IL-4 and anti-IL-12 blocking antibodies.

Control cells were differentiated in Th_1 polarising conditions containing 25ng/ml IL-12 and 5µg/ml anti-IL-4 blocking antibody. The individual reagents utilised are given in the table below (Table 35).

Table 35. Reagents used in culturing naive T cells during *in vitro* Th_1 and Th_{22} cell differentiation

Reagent	Company	Comment	ID	Concentration used
Rec. Human IL-6	Peprotech	Expressed in E.coli.	200-06	10ng/ml
Rec. Human TNFα	Peprotech	Expressed in E.coli.	300-01A	5ng/ml
Rec. Human IL-12	Peprotech	Expressed in E.coli.	200-12	25ng/ml
α-IL-4	Abcam	Clone 8F12	ab13780	5µg/ml
α-IL-12 (p40/p70)	BD Bioscience	Clone C11.5	554573	5µg/ml

Flow cytometry

T cells were stained and analysed as previously described (see Methods 2.1.1, page 80) using the FACS antibodies listed below (Table 36).

Table 36. FACS antibodies used in the assessment of Th_1 and Th_{22}-primed T cells

Name	Species	Company	Clone	ID	Concentration used
α-IL-17A-APC	Mouse IgG1	eBioscience	eBio64DEC17	17-7179-41	1/20
α-IL-22-PE	Mouse IgG1	R&D Systems	142928	IC7821P	1/20
α-IL-22-APC	Mouse IgG1	R&D Systems	142928	IC7821A	1/20
α-IFNγ-PE	Mouse IgG1	BD Bioscience	4S.B3	559326	1/20
α-IFNγ-V450	Mouse IgG1	BD Bioscience	B27	560371	1/20
Isotype-APC	Mouse IgG1	R&D Systems	11711	IC0041F	1/20
Isotype-PE	Mouse IgG1	BD Bioscience	MOPC-21	551436	1/20
Isotype-V450	Mouse IgG1	BD Bioscience	X40	642268	1/20

2.1.3. memory T cells

T cell enrichment using anti-fluorchrome beads

$5x10^7$ CD4 T cells were suspended in 500µl 1%BSA PBS containing 25µl anti-receptor antibody (Table 37) and was left at 4°C in the dark. After 10 minutes the cells were washed using 2ml of 1% BSA PBS and centrifuged at 1500rpm for 5 minutes. The resulting cell pellet was suspended in 500µl 1%BSA PBS containing 50µl anti-fluorchrome beads (Table 38) and left to incubate in the dark at 4°C for 15 minutes. The cells were washed as previously described and the supernatant taken off. The resulting cell pellet was suspended in 500µl 1%BSA PBS and magnetic bead isolation was carried out as previously described (see Methods 2.1.2, page 81).

Table 37. FACS Antibodies used in the analysis of novel Th_{22} surface receptor expression

Name	Species	Company	Clone	ID	Concentration used
α-FGFR2-FITC	Mouse IgG1	R&D Systems	98725	FAB684F	1/20
α-TIE1-PE	Goat IgG	R&D Systems	polyclonal	FAB619P	1/20
α-PDGFR-APC	Mouse IgG1	R&D Systems	PR7212	FAB1263A	1/20
α-CCR4-FITC	Mouse IgG2B	R&D Systems	205410	FAB1567F	1/20
α-CCR6-A488	Rat IgG2A	R&D Systems	140706	FAB590G	1/20
α-CCR10-APC	Rat IgG2A	R&D Systems	314305	FAB3478A	1/20
Isotype-FITC	Mouse IgG1	R&D Systems	11711	IC002F	1/20
Isotype-FITC	Mouse IgG2b	R&D Systems	133303	IC0041F	1/20
Isotype-A488	Rat IgG2A	R&D Systems	54447	IC006G	1/20
Isotype-PE	Goat IgG	R&D Systems	polyclonal	IC108P	1/20
Isotype-APC	Mouse IgG1	R&D Systems	11711	IC002A	1/20
Isotype-APC	Rat IgG2A	R&D Systems	54447	IC006A	1/20

Table 38. Anti-fluorchrome beads used in the isolation of surface receptor-positive T cells

Reagent	Company	ID
α-FITC Microbeads	Miltenyi Biotec	130-048-701
α-PE Microbeads	Miltenyi Biotec	130-048-801
α-APC Microbeads	Miltenyi Biotec	130-090-855

Validation of Receptor$^+$ T cell enrichment

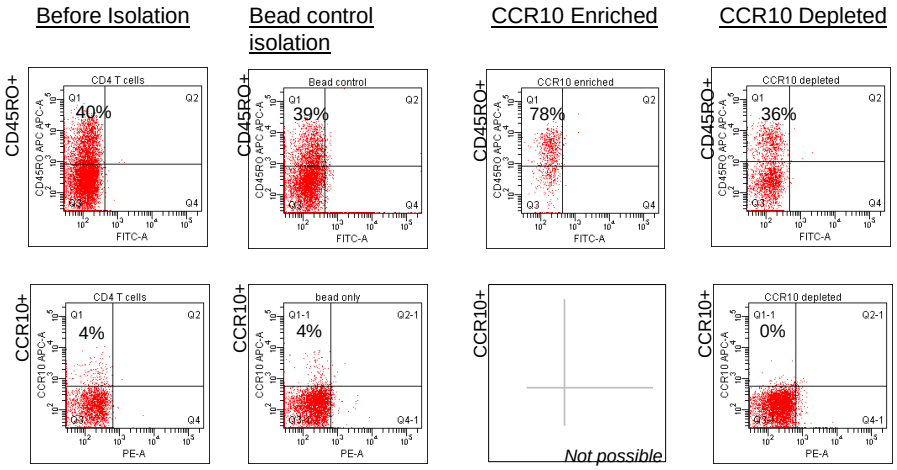


Figure 12. Flow cytometric analysis of CCR10 and CD45RO expression on peripheral blood T cells before and after CCR10 enrichment using anti-fluorochrome-coupled magnetic bead isolation.

FACS plots are representative

The flow cytometric analysis of CCR10-receptor-enriched T cells showed that CCR10 expression within the depleted fraction dropped from 4% to 0% (Figure 12). While CCR10 could not be stained in the enriched fraction, CD45RO expression increased from 40% to 78%. Isolation of Isotype stained and magnetically labelled T cells showed no enrichment in either CD45RO or CCR10 positive T cells.

Flow cytometry

The cells were stained and analysed as previously described (see Methods 2.1.1, page 80) using FACS antibodies listed below (Table 39).

Table 39. FACS antibodies used in the assessment of Th_{22} receptor enriched T cells

Name	Species	Company	Clone	ID	Concentration used
α-IL-17A-A488	Mouse IgG1	R&D Systems	41802	FAB3171G	1/20
α-IL-22-APC	Mouse IgG1	R&D Systems	142928	IC7821A	1/20
α-CD45RO-FITC	Mouse IgG2a	BD Bioscience	UCHL1	555492	1/20
α-CD45RO-APC	Mouse IgG2a	BD Bioscience	UCHL1	559865	1/20
Isotype-FITC	Mouse IgG2a	R&D Systems	133303	IC0041F	1/20
Isotype-A488	Mouse IgG1	R&D Systems	G94-56	554008	1/20
Isotype-APC	Mouse IgG1	R&D Systems	11711	IC002A	1/20

In vitro culture of T cells in the presence of putative Th_{22} receptor-ligands

CD4$^+$ T cells were activated using CD3/CD28 microbeads (Miltenyi) at a ratio of 1 bead to 4 cells and cultured in 5% human AB serum containing RPMI1640 substituted with FGF-1, -5, -7, or PDGF-BB (Table 40). Cultured T cells were obtained after 24, 48, and 72 hours for RT-PCR analysis.

Table 40. Recombinant FGF and PDGF proteins used in CD4 T cell co-culture

Reagent	Company	Comment	ID	Biological Conc.	Concentration used
Rec. Human FGF-1 (acidic)	Peprotech	Expressed in E.coli.	100-17A	0.5-10ng/ml	5 ng/ml
Rec. Human FGF-2 (basic)	Peprotech	Expressed in E.coli.	100-18B	0.1-0.5ng/ml	0.25 ng/ml
Rec. Human FGF-5	Peprotech	Expressed in E.coli.	100-34	<0.5ng/ml	0.25 ng/ml
Rec. Human PDGF-BB	ProSpec	Expressed in E.coli.	CYT-501	1-3ng/ml	2 ng/ml

Real-time-PCR

RNA isolation

RNA isolation was carried out using the Qiagen RNA isolation kit (Table 41). Between $2.5x10^5$-$5x10^5$ T cells (or tissue cells) were suspended in RLT buffer (Qiagen RNeasy Kit) containing 2-β-mecaptoethanol (diluted 1/100) and immediately frozen and stored at -20°C. After a maximum of 14 days, samples were defrosted, transferred into a Qiagen shredder spin tube and centrifuged at 13000rpm for 2 min using an Eppendorf 4075 bench centrifuge. After centrifugation an equal volume of 70% ethanol was added to the homogenised lysate and mixed by pipetting. The mixture was then transferred to RNA spin columns and briefly centrifuged for 15 seconds at 10000rpm. 700µl of RW1 buffer was added and centrifuged again for 15 seconds at 10000rpm. 500µl RPE buffer was then added to the spin column and centrifuged for 15 sec at 1000rpm. The step was repeated using a longer centrifugation of 2 minutes. The spin column was then centrifuged empty for 1 minute at full speed to remove any remaining liquid. The column was then placed into a fresh eppendorf tube and 30µl of dH_2O was added. The RNA was eluted by centrifugation for 1 minute at 10000rpm and stored at -80°C.

Table 41: RNA isolation reagents utilised throughout the study

Name	Company	ID	Containing
Shredder/Homogeniser	Qiagen	79656	Homogenising spin columns
RNeasky kit	Qiagen	740106	RNA Spin columns
"	"	"	RLT Buffer (+2βME)
"	"	"	RW1 Buffer
"	"	"	RPE Buffer (+ethanol)

Reverse transcription

The cDNA synthesis was carried out using the RevertAid First Strand cDNA Synthesis Kit (Fermentas K1621). Before the sample RNA was reverse transcribed, the quality and quantity of the RNA was assessed using a Nanodrop analyser. 1µg of sample RNA was added to the cDNA reaction mixture (Table 42) and added to a benchtop PCR machine (Sensoquest Labcycler) programmed to the recommended cycling times and temperatures (Table 43). The resulting cDNA was diluted 1:5 before use.

Table 42. Composition of the cDNA reaction mixture (20µl)

Amount/Volume	Reagent (from Fermentas ReverseAid kit)
4 µl	5X cDNA Buffer
2 µl	dNTPs
1 µl	Hexamere Primers
0.5 µl	Rnase Inhibitor
1 µl	RT Enzyme
11.5 µl	dH_2O containing 1ug Target RNA

Table 43. PCR reaction setup used in the conversion of RNA to cDNA

Step	Time	Temperature
1	10 minutes	25°C
2	60 minutes	42°C
3	10 minutes	70°C
4	∞	4°C

RT-PCR reaction and analysis

All primers were tested before use. Using serial diluted cDNA each primer pair produced a linear amplification pattern ($r^2 \geq 0.95$). None of the primers produced a signal in the absence of cDNA. In addition the dissociation (or melting) curve was analysed and nonspecific amplification was further eliminated by excluding ct values above 30.

The RT-PCR reaction was carried out using SYBR green detection. Using an automated robot (Eppendorf Epimotion 5075) 3.4µl of 1/5 diluted cDNA and 6.6µl of Primer/SYBR green mix (Table 44) was pipetted in triplicate into a 384-well clear optical reaction plate (AB 4309849). The plate was covered with a clear adhesive cover (AB MicroAmp 4311971) and sealed. The RT-PCR reaction was carried out using an AB 7900HT RT-PCR machine programmed using the SDS 2.3.Sequence Detection System software. Please refer to table 42 for individual primer sequences used.

Table 44. RT-PCR Primer and SYBR green reaction mixture

Amount/Volume	Reagent
75µl	Power SYBR Green (AB 4367659)
1µl	100uM Forward Primer
1µl	100um Reverse Primer
23µl	dH_2O

Table 45. RT-PCR primer sequences used to analyse PDGFR enriched and depleted T cells

Gene	Primer	5'-3' DNA sequence	Baseline Ct (Media Control)
IFNγ	Forward	TCT CGG AAA GGA TGA AAT ATA CAA GTT	19-20
	Reverse	GTA ACA GCC AAG AGA ACC CAA	
IL-4	Forward	AAA CGG CTC GAC AGG AAC CT	27-29
	Reverse	ACT CTG GTT GGC TTC CTT CAC	
IL-5	Forward	GCT CTT GGA GCT GCC TAC GT	26-27
	Reverse	AAG GTC TCT TTC ACC AAT GCA CT	
IL-9	Forward	ATC CTG GAC ATC AAC TTC CTC ATC	27-28
	Reverse	CAG AGA CAA CTG GTC ACA TTA GCA	
IL-13	Forward	GAT TCC AGG GCT GCA CAG TA	23-26
	Reverse	GGT CAA CAT CAC CCA GAA CC	
IL-17A	Forward	CCA TCC CCA GTT GAT TGG AA	24-25
	Reverse	CTC AGC AGC AGT AGC AGT GAC A	
IL-22	Forward	GGC CAG GCT CAG CAA CAG	19-20
	Reverse	CCT CTG GAT ATG CAG GTC ATC AC	
TGFβ1	Forward	AAA TTG AGG GCT TTC GCC TTA	22-23
	Reverse	GAA CCC GTT GAT GTC CAC TTG	
IL-10	Forward	TGC CTA ACA TGC TTC GAG ATC TC	25-26
	Reverse	GTT GTC CAG CTG ATC CTT CAT TT	
PDGFA	Forward	ACG GGG GCC AGA TCA GGA AG	31-32
	Reverse	AAT TTC GCC GCC ACA GGA GA	
PDGFB	Forward	GCG CTC TTC CTG TCT CTC TG	30-31
	Reverse	TCG AGT GGT CAC TCA GCA TC	
18S	Forward	CAG CCA CCC GAG ATT GAG CA	9-10
	Reverse	TAG TAG CGA CGG GCG GTG TG	

The resulting data was checked for errors and the raw ct values were exported into Microsoft excel files. The relative expression of each gene was calculated using the ΔΔct method using 18s as a housekeeping gene (see below).

$$2^{-((ct\ Target\ Gene - ct\ Housekeeping\ Gene\ [sample]) - (ct\ Target\ Gene - ct\ Housekeeping\ Gene\ [media\ control]))}$$

$$= 2^{-(\Delta ct\ sample - \Delta ct\ media\ control)}$$

$$= 2^{-\Delta\Delta Ct}$$

For example:

$$2^{-((18.1ct\ IFN\gamma - 9.2ct\ 18s\ [sample]) - (24.3ct\ IFN\gamma - 9.3ct\ 18s\ [media\ control]))}$$

$$= 2^{-(8.9-15)}$$

$$relative\ fold\ change = 68.59$$

2.2. from human asthmatic bronchial mucosa

Participants

Participants were recruited from patients at the Asthma Unit of the Brompton Hospital. All recruited participants were previously diagnosed as asthmatics and qualified for the study by having no additional health problems. Steroid use was not prohibited but recorded. After taking written informed consent tissue biopsies were taken during a fiberoptic bronchoscopy by medical staff at the Brompton Hospital. The bronchial tissue was immediately placed into

a falcon tube containing sterile RPMI media (incl. penicillin and streptomycin) briefly stored at 4°C for 30-45 minutes before being transferred to the laboratory. Note: For more detail on individual patients refer to appendix (Table 70, page **Error! Bookmark not defined.**).

Creation of lung T cell lines

The bronchial tissue was centrifuged three times at 2000rpm for 5 minutes, with media changes in between. The bronchial biopsy was then placed into a 48-well plate and left overnight at 37°C. The next day the biopsy was transferred together with fresh media into a CD3/CD28 (0.5µg/ml; R&D MAB100, MAB342) pre-coated well. After one week the tissue was discarded and the media centrifuged at 2000rpm for 5 minutes. The resulting cell pellet was suspended in fresh RPMI media containing 20ng/ml IL-2 (Peprotech) and transferred to a CD3/CD28 pre-coated well and placed back in the incubator. After a further 14 days a small aliquot of cell suspension was taken and analysed by FACS for CD3, CD4 and CD8 expression.

Creation of lung T cell clones

Lung T cell lines were diluted in cloning media (Table 46) to a concentration of 1.75-3x10^5 cells/ml. This cell suspension was further diluted using a dilution series (Table 47) and 100µl of cell suspension (8 cells/ml) was added per well to a clear round bottom 96 well plate resulting in a theoretical concentration of 0,8 cells per well.

To maintain and stimulate T cell expansion during cloning, irradiated feeder cells were used (e.g. PBMCs from a different donor). These cells were counted and reconstituted at 2x10^6 cells/ml cloning medium. After irradiation at 4000rads the cells were adjusted to a total concentration of 3.5x10^5 cells/ml and 2% PHA and 40ng/ml IL-2 were added. 100µl of the feeder cell solution was then added to each well in the round bottom 96-well plate.

Table 46. Components of the T cell cloning media

Amount/Volume	Reagent
50ml	Fetal Bovine Serum (FBS)
25ml	Human Serum (Invitrogen H4522)
5ml	200mM Glutamin (Invitrogen 25030-024)
5ml	100x Antibiotic/-mycotic Solution (PAA P11-002)
5ml	100x Non-essential amino acids (Invitrogen 11140-35)
5ml	100mM Sodium-Pyruvate (Invitrogen 11360-039)
0.5ml	50mM 2β-mercaptoethanol (Sigma)
404,5ml	RPMI (Invitrogen 31870-025)

Table 47. Serial dilution setup employed in the generation of T cell clones

Step	Dilution	Final concentration	Determined by
A	1:2, 1:4, 1:8, 1:16...	1.75-3x10^5 cells/ml	Haemocytometer
B	1:10 followed by	1750-3000 cells/ml	Theoretical
	1:10	175-300 cells/ml	Theoretical
C	400 cells into 50ml	8 cells/ml	Theoretical
	100µl per well	0,8 cells/well	Theoretical

Maintenance & T cell clone expansion

Due to the long term culture extreme care was taken to disinfect incubators, tissue culture hoods and equipment on a regular basis. On day 7 100µl of media was removed and 100µl of new irradiated feeder cell suspension was added and from now onwards changed every second day.

From day 10 T cell clones could be spotted as pear shaped as watery, shiny cells overgrowing the feeder cell pellet. Potential clones were split into two new 96 wells and new 100µl cloning medium containing 40ng/ml IL-2 was added. The media was changed every second day until the cells reached 70% confluence. At that stage these cells were transferred into a larger well in a 24 well plate. IL-2 was increased by 20ng/ml steps to 60ng/ml and 80ng/ml if the cell proliferation appeared to arrest. After cell numbers increased to sufficient levels a small aliquot was taken and assessed for intracellular cytokine production by flow cytometry. See the table below for an overview of the individual steps involved (Table 48).

Table 48. Individual steps in expanding T cell clones over time

Day	Setup/Maintenance	Comment
1	Setup of serial T cell dilution & first feeder cell stimulation +PHA +IL-2	
7	Re-stimulation with new feeder cells +PHA +IL-2	
9	Remove 100µl media and replace with new media +IL-2	
11	Remove 100µl media and replace with new media +IL-2	Look for visible clones
14	Remove 100µl media and replace with new media +IL-2	Look for visible clones/Expansion
15	Remove 100µl media and replace with new media +IL-2	Expansion
17	Remove 100µl media and replace with new media +IL-2	Expansion
19	Remove 100µl media and replace with new media +IL-2	Expansion
21	Re-stimulation with new feeder cells +PHA +IL-2	Expansion
..	Media change (every second day) Feeder cells (every 14days)..	Expansion

Flow cytometry of T cell lines and clones

Polyclonal T cell stimulation, fixation, staining, and acquisition were carried out as previously described (see Methods 2.1.1, page 80) using FACS antibodies listed below (Table 49).

Table 49. FACS antibodies used in the characterisation of T cell lines and clones

Name	Species	Company	Clone	ID	Concentration used
α-(αβ)TCR-FITC	Mouse IgG1	BD Bioscience	WT31	333140	1/20
α-(γδ)TCR-FITC	Mouse IgG1	BD Bioscience	B1	561995	1/20
α-CD4-PERCP	Mouse IgG1	BD Bioscience	L200	550631	1/20
α-CD8-APC H7	Mouse IgG1	BD Bioscience	SK1	560273	1/20
α-IFNγ-V450	Mouse IgG1	BD Bioscience	B27	560371	1 in 20
α-IL-4-PE	Mouse IgG1	BD Bioscience	8D4-8	562046	1/20
α-IL-17A-A488	Mouse IgG1	R&D Systems	41802	FAB3171G	1/20
α-IL-22-PE	Mouse IgG1	R&D Systems	142928	IC7821P	1 in 20
α-IL-22-APC	Mouse IgG1	R&D Systems	142928	IC7821A	1/20
Isotype-FITC	Mouse IgG1	R&D Systems	11711	IC002F	1/20
Isotype-APC H7	Mouse IgG1	BD Bioscience	MOPC-21	560167	1/20
Isotype-V450	Mouse IgG1	BD Bioscience	X40	642268	1 in 20
Isotype-PE	Mouse IgG1	BD Bioscience	MOPC-21	551436	1/20
Isotype-A488	Mouse IgG1	R&D Systems	G94-56	554008	1/20
Isotype-APC	Mouse IgG1	R&D Systems	11711	IC002A	1/20

3. IL-22 in human tissue regeneration and repair

3.1. IL-22 Receptor expression in human lung cell lines

Study design and primary lung cell lines

Lung cell lines

All structural cell lines were obtained from healthy non-asthmatic donors. Bronchial epithelial and pulmonary fibroblast cell lines were obtained from Lonza while airway smooth muscle cell lines were kindly provided by Dr. Paul Lavender from Kings College London. Lung epithelial cells and lung fibroblasts were classified by Lonza based on questionnaire. Both donors reported no respiratory related disorders including Asthma, COPD or Cystic Fibrosis. All three donors of smooth muscle tissue had no respiratory related disease, did not smoke and showed no abnormal spirometry (FEV_1) or methacholine (PC20) measurements. Aliquots of early passage cells were made, frozen in 15% Dimethyl sulfoxide (DMSO) 50% serum containing media and stored in liquid nitrogen and otherwise grown until passage 5-6. See table below for individual cell lines used (Table 50).

Table 50. Description of primary structural cell lines studied

Cell type	Culture	Gender	Age	Status	ID
Bronchial Epithelium	Primary	M	43	Healthy Non-Asthmatic	Lonza 7F4167
Pulmonary Fibroblasts	Primary	M	45	Healthy Non-Asthmatic	Lonza 120188
Airway Smooth Muscle	Primary	n.a.	n.a.	Healthy Non-Asthmatic, Normal FEV_1 & PC20	KCL-PL-HB129
Airway Smooth Muscle	Primary	n.a.	n.a.	Healthy Non-Asthmatic, Normal FEV_1 & PC20	KCL-PL-HB138
Airway Smooth Muscle	Primary	n.a.	n.a.	Healthy Non-Asthmatic, Normal FEV_1 & PC20	KCL-PL-HB139

In vitro culture

Cells were cultured in dedicated cell culture media in the presence or absence of various cytokines (Table 51) over time. For epithelial cells Bronchial Epithelial Cell Growth Medium

(BEGM Bulletkit; Lonza), for fibroblasts cells Fibroblast Growth Medium-2 containing 2% serum (FGM-2; Lonza) and for smooth muscle cells Dulbecco Modified Eagle Medium (DMEM; Gibco) supplemented with 10% FCS, 4 mmol/L l-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2.5 µg/mL amphotericin B media was used. Cells were detached using TrypLE (Invitrogen 12604-021).

Table 51. Individual cytokines used in the stimulation of structural cell lines *in vitro*

Name	Company	ID	Concentration used
Rec. human IFNγ	Peprotech	300-02	10ng/ml (variable)
Rec. human TNFα	Peprotech	300-01A	10ng/ml (variable)
Rec. human IL-4	Peprotech	200-04	50ng/ml (variable)
Rec. human IL-5	Peprotech	200-05	50ng/ml (variable)
Rec. human IL-9	Peprotech	200-09	50ng/ml (variable)
Rec. human IL-13	Peprotech	200-13	50ng/ml (variable)
Rec. human IL-17A	Peprotech	200-17	50ng/ml (variable)
Rec. human IL-22	R&D Systems	782-IL-010/CF	50ng/ml (variable)
Rec. human TGFβ1	R&D Systems	240-B-002	10ng/ml (variable)
Rec. human IL-10	Peprotech	200-10	50ng/ml (variable)

Western Blotting

One T25 flask of adherent cells was lysed in protein lysis buffer (Table 52). The lysate was subjected to repeated cycles of freeze thawing using liquid nitrogen and a water bath at 37°C. After 3 cycles the lysate was centrifuged at 13000rpm for 30 minutes using a bench top Eppendorf 5424 centrifuge. The soluble fraction was removed and stored at -80°C.

Table 52. Composition of the protein lysis buffer

Amount/Volume	**Reagent**
132 µl	Aprotonin from bovine lung (Sigma A6279)
10 µl	Igepal CA-630 (Sigma I3021)
10 µl	Phenylmethanesulfonyl fluoride solution (Sigma/Fluka 93482)
4 µl	Iodoacetate solution (Sigma A3221)
4 µl	EDTA (Gibco 15575-038)
10 µl	Protease inhibitor cocktail (Roche)
830 µl	1X PBS

Determining Protein Concentration (BCA assay)

The total protein concentration in each cell lysate was measured using the Bicinchoninic acid (BCA) assay (Pierce BCA Protein Assay Kit 23225). Bovine serum albumin standards (20-2000pg/ml) were used to determine a standard curve of protein concentrations. The assay itself was carried out in a standard clear 96-well plate. In brief:

The BCA working reagent was prepared fresh by mixing 50 parts BCA reagent A to 1 part BCA reagent B. 200µl of the mixture was then pipetted into the 96-well plate. Then 25µl of unknown cell lysate or BSA standard was pipette in triplicate into the 96-well plate. The plate was covered and left in a tissue incubator at 37°C for 30 minutes. After the incubation the plate was briefly cooled to room temperature. The absorbance of each well (at 562nm) was

determined using a spectrophotometer (Teacan Sunrise plate reader & Magellan Software). The raw data was exported to Microsoft excel and the standard curve determined. Using the equation of the line derived from the linear part of the standard curve cell lysates protein concentrations were determined.

Equation of the line:

$$\frac{(Absorbance - Intercept)}{Slope}\ x\ Dilution\ Factor = Protein\ Concentrations$$

For example:

$$\frac{(0.510\ OD[562nm] - 0.1711)}{0.001019}\ x\ 10 = 3.326\ \mu g/\mu l$$

SDS-PAGE & Transfer to nitrocellulose membrane

50µg of protein in a total volume of 20µl containing 5µl loading buffer (Table 53) was loaded onto a 4%-12% Bis-Tris Gel (Novex NP0323) inside a gel tank (Invitrogen X cell Surelock) containing MES SDS running buffer (Invitrogen). For example; 7.5µl epithelial extract [e.g. of 3.326µg/µl protein lysate] + 5µl 4X loading buffer +7.5µl lysis buffer. In addition 5µl of Benchmark protein ladder (Invitrogen 107480-010) was loaded in the wells flanking the protein samples of interest. The Gel was run for 45 minutes at 200 Volt using 104mA.

Table 53. Composition of the SDS PAGE loading buffer (4x)

Amount/Volume	Reagent
2 ml	1M Tris-HCL (pH 6.8)
0.8 g	SDS (Sigma SDS7)
4 ml	10% Glycerol (G5516)
0.4 ml	14.7M 2-β-mercaptoethanol (Sigma M7154)
1 ml	0.5M EDTA (Gibco 15575-038)
8 mg	Bromophenol Blue (Sigma B0126)

The proteins were transferred from the Nupage Gel onto a 0.45um porse-size Nitrocellulose membrane (Invitrogen Lc2001) using an X cell II blotting module (Invitrogen). 10% Methanol and 0.1% NuPage Reducing agent (Invitrogen) was added to the transfer buffer and the electrophoresis was carried out using 30 V for 1 hour.

Western Blotting and detection

The membrane was blocked for 1 hour in 0.1% Tween-20 5% dried skimmed milk (Marvel) containing PBS. The membranes were rinsed 3 times in PBS. The primary antibodies (e.g. goat-anti human IL-22RA2 and mouse anti alpha tubulin) diluted in 5% dried skimmed milk (Marvel) PBS were added to the membrane. The membrane was left overnight at 4°C on a platform shaker (Sturat Scientific) at 20 rpm. After three PBS rinses the secondary antibody

(e.g. donkey anti goat IgG) was added to the membrane for 1 hour. After 3 additional PBS rinses the membrane was incubated with the other secondary antibody (e.g. goat anti mouse IgG). After 3 more washes the membrane was incubated in the dark with activated ECL reagent (GE Health ECL$^+$ RPN2132). Chemilumiescence was detected on GE hyperfilm (28906825). The ECL film was developed manually using the two component Agfa medical X-ray film developer G153. The film was left for 30 seconds in the pre-prepared developer (1l Sol A, 0.35l Solution B, 1,5l dH_2O) and then fixed for a 30 seconds using pre-prepared Agfa Rapid Fixer G353 (0.5l Stock sol, 1.5l dH_2O). The developed films were rinsed, dried and marked for the location of the protein ladder. See the table below for individual antibodies and concentrations used (Table 54).

Table 54. Antibodies used in the analysis of IL-22 receptor expression by Western Blot analysis

Name	Species	Company	ID	Concentration used
α-IL-22RA1	Mouse monoclonal	R&D systems	MAB2770	1/500 (1µg/ml)
α-IL-22RA2 (T16)	Goat polyclonal	Santa Cruz Biotechnology	Sc64640	1/100 (2µg/ml)
α-alpha-tubulin	Mouse monoclonal	eBioscience	14-4502-80	1/1000 (0.5µg/ml)
α-mouse Ig –HRP	Goat polyclonal	Dako	P044701	1/1000
α-goat Ig – HRP	Donkey polyclonal	Santa Cruz Biotechnology	sc-2020	1/5000

Flow cytometry

Fc receptor blocking solution (Miltenyi 120-000-442) was staining using the antibodies listed in the table below (Table 55). The flow cytometric staining was carried out as previously described (see Methods 2.1.1, page 80). Before the cells were acquired the cell suspension was strained through cell pre-separation filters (Miltenyi 130-041-407).

Table 55. FACS antibodies used in the analysis of IL-22RA1 surface expression

Name	Species	Company	Clone	ID	Concentration used
α-IL-22RA1-APC	Mouse IgG1	R&D Systems	305405	FAB2770A	1/20
Isotype-APC	Mouse IgG1	R&D Systems	11711	IC002A	1/20

RT-PCR

RNA isolation, cDNA transcription and RT-PCR acquisition was carried out as described previously (see Methods 2.1.3, page 86) using RT-PCR primer sequences listed below (Table 56).

Table 56. RT-PCR primer sequences used in studying IL-22 receptor regulation in structural cell lines

Gene	Primer	5'-3' DNA sequence	Baseline Ct (Media Control)
IL-22RA1	Forward	CCC TAC ATG TGC CGA GTG AA	27-28
	Reverse	CCG GAG AAG GAG TAG GTC CAT	
IL-22RA2	Forward	TTA CCA AAA GGA AGA CAG CAT CTG	29-30
	Reverse	GAC TGT TCA GGC AAC CAG TGT TC	
18S	Forward	CAG CCA CCC GAG ATT GAG CA	5-6
	Reverse	TAG TAG CGA CGG GCG GTG TG	

3.2. Effect of IL-22 on bronchial epithelial cells

3.2.1. Wound healing

Wound healing assay

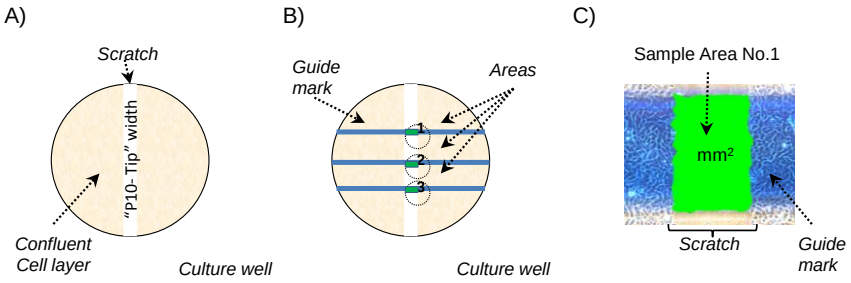


Diagram 4. Analysing epithelial cell wound closure

A) Full confluent epithelial cell layers are injured using a P10 pipette tip (3 wells per condition). B) 3 Guide marks on the bottom of each well intersect the scratch and facilitate the relocation of identical analysis areas over time (3 per well). C) The area investigated is highlighted in green. Its calculated surface area is set to 100% at time point 0 (maximum injury).

The assay was designed using a normal 12-well tissue culture plate. Guide marks were drawn on the bottom of the plate using a water-resistant blue pen (Diagram 4B). Within each culture well bronchial epithelial cells were cultured until 90%-95% confluence was reached. Using a p10 pipette tip and a ruler a scratch was made in the middle of each confluent layer (Diagram 4A). Cells were then grown in the presence or absence of IL-22. At each time point images were taken of each sample area (Diagram 4C) using a digital camera. The images were then analysed using imaging software (Motic Images Plus 2.0) and the changes in surface area calculated.

Reproducibility of the wound healing assay

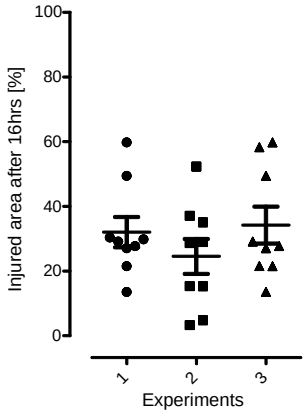


Figure 13. Inter and intra-experimental variability of the wound healing assay

Represented are 3 experiments which were carried out on different days. Within each experiment 9 data points were collected and are represented . Represented are Mean and Standard deviation. Coefficient of variation Exp.1(43.9%), Exp.2(65.8%), Exp.3(49.9%). Coefficient of variation between experiments 1-3 (16.7%).

The results showed that the remaining injured area varied greatly within one experiment from less than 20% to ca. 60% (Figure 13). A comparison of 3 experiments carried out on different days of the week (Figure 13, Exp.1-3) showed that the intra-assay variation was much lower and within an acceptable range.

Cell Cycle analysis /Proliferation

Cell cycle analysis was carried out using propidium iodide (PI). In brief: Epithelial cells were cultured for 24 hours under normal conditions using 10ng/ml TGFβ1, 200ng/ml IL-22 or media control. Cells were detached using TrypLE and counted on a haemocytometer. $2x10^6$ cells were added to individual FACS tubes and centrifuged for 5 minutes at 2000rpm. The media was removed and the cell pellet was suspended drop wise under continuous vortexing in 1ml ice-cold 80% ethanol. The cells were left on ice for 1 hour and washed twice with ice cold PBS. The cells were then re-suspended in 0.5ml PI staining solution (Table 57) and left to incubate on ice for 3 hours. After incubation the cell solution was strained through a filter (Miltenyi) removing large cell aggregates prior to FACS analysis.

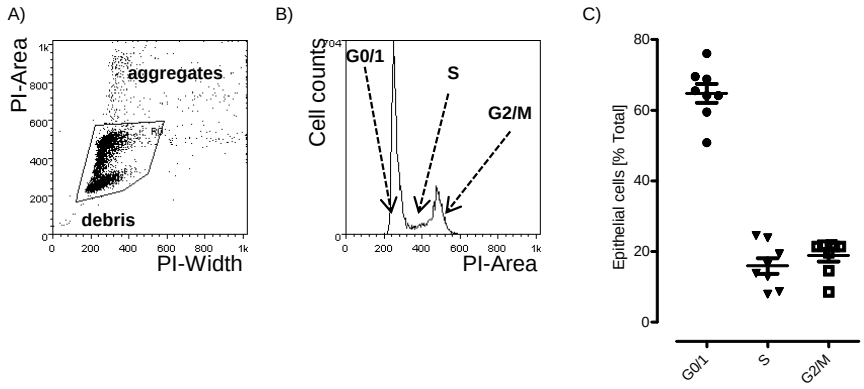


Figure 14. Analysis of individual bronchial epithelial cell cycle stages using propidium iodide

A) Cell gating strategy to exclude debris and aggregates B) Histogram of PI-Area identifying individual cell cycle stages (G0/1, S, and G2/M) C) Reproducibility of results of control cultured bronchial epithelial cells

Using a BD FACS Calibur machine cells were gated using channel FL3 fluorescence (Figure 14). By displaying PI-width and PI-area, debris (low PI-width and area) and aggregates (high PI-width and/or area) were excluded from further analysis through cell gating (Figure 14A). The gated cell population was then displayed using individual histograms of PI-area and its spectra divided into different cell cycle stages (Figure 14B). The resulting % of cells in G/01, S and G2/M-phase recorded and used for further analysis (Figure 14C).

Table 57. Composition of the Propidium Iodide staining solution

Amount/Volume	Reagent
0.1%	Triton X-100 (Sigma X100)
0.1 mM	EDTA (Gibco 15575-038)
50 µg/ml	Rnase A (Sigma R-4875)
50 µg/ml	Propidium Iodide (Sigma P-4170)

RT-PCR

Human bronchial epithelial cells were cultured in the presence or absence of cytokines (Table 58). Bronchial epithelial RNA isolation, subsequent cDNA transcription and RT-PCR acquisition was carried out as previously described (see Methods 2.1.3, page 86) using RT-PCR primer pairs listed below (Table 59).

Table 58. Recombinant cytokines used in the bronchial epithelial wound healing assay

Gene	Company	ID	Concentration used
Rec. human IL-22	R&D Systems	782-IL-010/CF	50ng/ml (variable)
Rec. human IFNγ	Peprotech	300-02	10ng/ml (variable)

Table 59. RT-PCR primer sequences used in the assessment of bronchial epithelial gene expression

Gene	Primer	5'-3' DNA sequence	Baseline Ct (Media Control)
ICAM1	Forward	AGG GTA AGG TTC TTG CCC AC	24-26
	Reverse	TGA TGG GCA GTC AAC AGC TA	
IL-6	Forward	TGG CTG AAA AAG ATG GAT GC	28
	Reverse	GAT GAT TTT CAC CAG GCA ACT CT	
IL-7	Forward	TCA TTA TTC AGG CAA TTG CTA CC	29-30
	Reverse	TGA AGG TAA AGA TGG CAA ACA A	
IL-15	Forward	TCC ACG ATG CCT CCT ACA A	29-30
	Reverse	TGT TCC ATC ATG TTC CAT GC	
CCL2	Forward	AGG TGA CTG GGG CAT TGA T	33-34
	Reverse	GCC TCC AGC ATG AAA GTC TC	
CCL5	Forward	TGT ACT CCC GAA CCC ATT TC	32-33
	Reverse	TAC ACC AGT GGC AAG TGC TC	
CXCL8	Forward	TTG GCA GCC TTC CTG ATT TC	27-28
	Reverse	AAC TTC TCC ACA ACC CTC TG	
CXCL9	Forward	TCA CAT CTG CTG AAT CTG GG	31-33
	Reverse	CCT TAA ACA ATT TGC CCC AA	
CXCL10	Forward	GCT GAT GCA GGT ACA GCC T	33-34
	Reverse	CAC CAT GAA TCA AAC TGC GA	
CXCL11	Forward	ATG CAA AGA CAG CGT CCT CT	29-30
	Reverse	CAA ACA TGA GTG TGA AGG GC	
MMP1	Forward	AGG TGG ACC AAC AAT TTC AGA GA	25-26
	Reverse	AAG AGA ATG GCC GAG TTC ATG A	
MMP3	Forward	TGA ACA ATG GAC AAA GGA TAC A	28-29
	Reverse	TTT CAT GAG CAG CAA CGA GAA	
MMP10	Forward	CTG ATC GGC CCA GAA CTC ATT	26
	Reverse	ATT TTG GCC CTC TCT TCC AT	
TGFα	Forward	GGG CAG TCA TTA AAA TGG GA	21-22
	Reverse	GCT CTG GGT ATT GTG TTG GC	
EGF	Forward	TGG TTC CTT CTG TGT CAA TCC	32-34
	Reverse	GTA CTC CGC AGG AAA TGG G	
EGFR	Forward	TCC TCT GGA GGC TGA GAA AA	20-21
	Reverse	GGG CTC TGG AGG AAA AGA AA	
18S	Forward	CAG CCA CCC GAG ATT GAG CA	8-9
	Reverse	TAG TAG CGA CGG GCG GTG TG	

Results

1. IL-22 expression in allergic inflammation

To determine the expression of Interleukin-22 and its receptors during an allergic reaction, biological samples from two human allergen challenge models were analysed. These included a local bronchial segmental allergen challenge and an intradermal allergen skin challenge.

1.1. Local bronchial segmental allergen challenge

In this first study, a human bronchial segmental allergen challenge was performed (see Method 1.1, page 70) mimicking a natural exposure of allergen to the lower airways.

1.1.1. Demographics of study participants

Table 60. Clinical data of allergic asthmatic and healthy non-atopic controls who underwent fiberoptic bronchoscopy with segmental allergen challenge

Treatment Group	Patients (n=15)	Controls (n=5)
Age (Y)	32.5 (2.0)	23 (2.4)*[1]
Gender (M/F)	7/8	3/0*[1]
Challenged (Grass/HDM)	10/5	0/5
Multiple atopy (Yes/No)	7/8	None
Total IgE (IU/ml)	358.3 (74.2)	<40
RAST (IU/ml)	GP:62.2 (10.5) HDM: 38.1 (17.9)	<0.35 <0.35
PC 20 (mg/ml)	3.5 (1.4)*[2]	>32
FEV_1 % predicted	109.8 (4.8)	>100
% Fall in FEV_1	25.3 (2.4)	≤ 8

*[1] Age and Gender for 2 control subjects (Ac18, Ac23) were missing. *[2] PC20 data is represented in log scale.
If not otherwise described, data is represented as Mean (SEM=Standard Error of the mean).

20 individuals were recruited of whom 15 were allergic asthmatics and 5 normal healthy controls (Table 60). Asthmatics and normal healthy controls were matched for their age and gender. Compared to the normal healthy controls, allergic asthmatics had significantly higher total serum IgE (p=0.001). In addition, allergic asthmatics had high levels of specific IgE to the inhaled allergen (RAST; p=0.001) and raised bronchial responsiveness to methacholine inhalation challenge (Methacholine PC20; p=0.008).

1.1.2. Spirometry and Bronchoalveolar lavage eosinophils

Spirometry

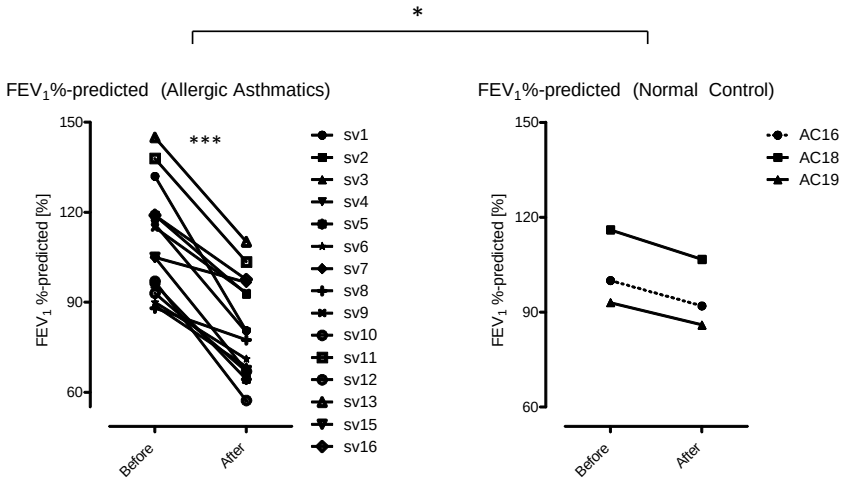


Figure 15. Percentage-predicted forced expiratory volume (FEV_1) before and after segmental allergen challenge

Analysis: * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001 (Before vs. After: Wilcoxon; Allergic Asthmatic vs. Normals: Mann-Witney U-test)

To assess a positive physical reaction towards the allergen, the reduction in lung function by measuring the 1 second forced expiratory volume (FEV_1) was determined. Compared to normal healthy controls, asthmatics showed a significant reduction in lung function after allergen challenge by 25.3 (2.4)% points in FEV_1-% predicted (p=0.013) (Figure 15).

Bronchoalveolar lavage eosinophils

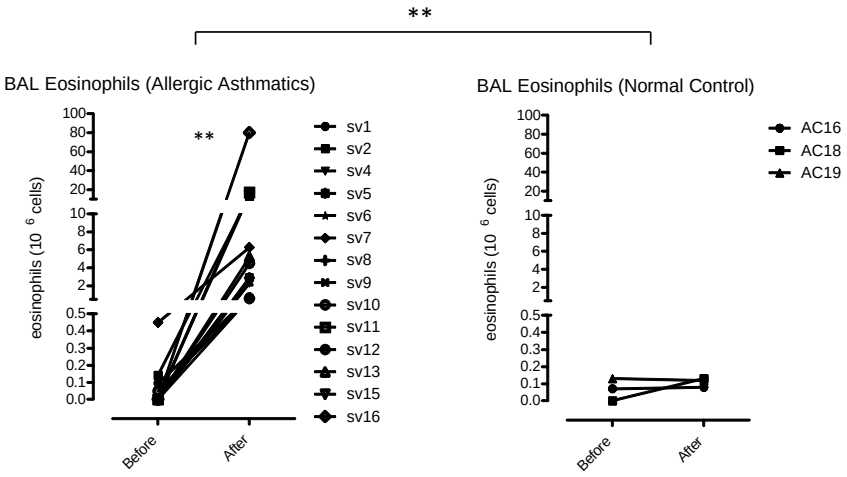


Figure 16. Total eosinophil counts (10^6) in bronchoalveolar-lavage (BAL) before and after segmental allergen challenge

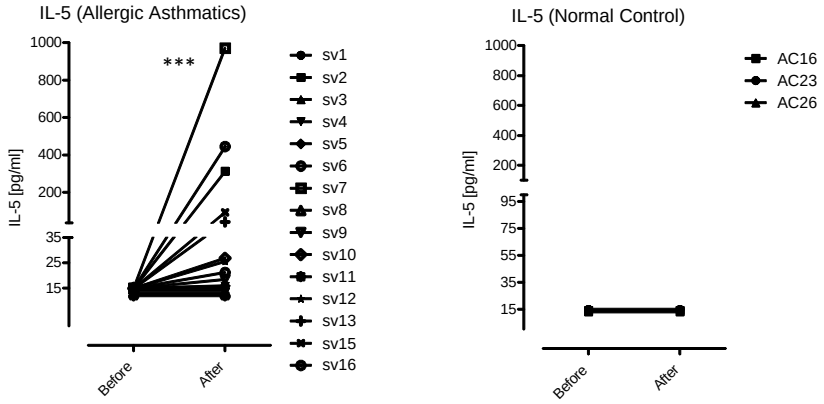
Analysis: * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001 (Before vs. After: Wilcoxon; Allergic Asthmatic vs. Normals: Mann-Witney U-test)

As a positive cellular marker of an allergic immune response, the numbers of eosinophils in bronchoalveolar lavage before and after allergen challenge were determined. The analysis showed that only allergic asthmatics had increased numbers of eosinophils in their bronchoalveolar lavage after challenge. In these asthmatics, eosinophil numbers increased 333-fold from $0.03x10^6$(0.1) before to $10x10^6$(1.2) after challenge (p=0.001) (Figure 16).

1.1.3. Cytokine expression in Bronchoalveolar lavage

IL-5 and IFNγ

A) Interleukin-5



B) Interferon-γ

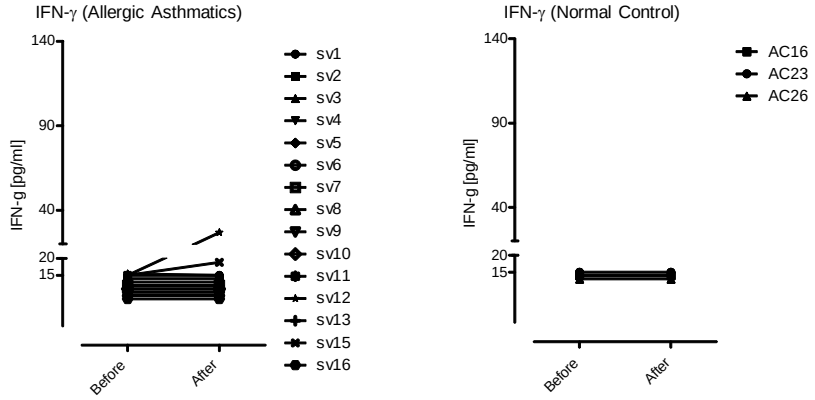


Figure 17. IL-5 and IFNγ cytokine ELISA measurements in (BAL) before and 24 hrs after segmental allergen challenge

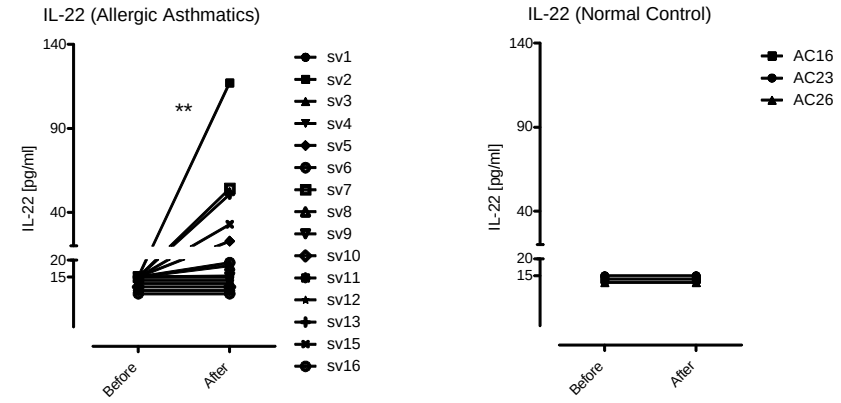
Analysis: * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001 (Before vs. After: Wilcoxon; Allergic Asthmatic vs. Normals: Mann-Witney U-test)

During an allergic immune response, Th_2 -type cytokines increase in expression while Th_1 –type cytokines remain low or absent. To validate that the BAL could reflect the underlying Th_2–type immune response, IL-5 and IFNγ concentrations were determined before and after allergen challenge by ELISA.

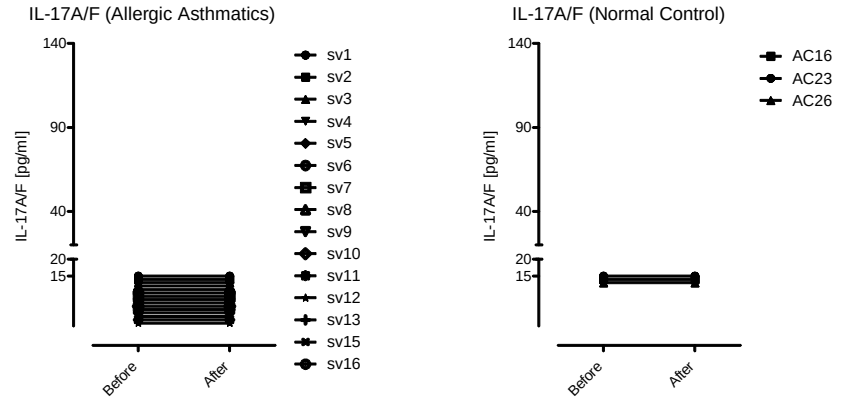
The ELISA results showed that after allergen challenge IL-5 increased in 11/15 asthmatics but none of the normal healthy controls (Figure 17). BAL IL-5 increased from less than 15

pg/ml to 134.2(4.2) pg/ml (p=0.001). The results of the IFNγ ELISA showed increased expression in 2/15 asthmatics and none of the normal healthy controls. The increase of IFNγ in allergic asthmatics was not found to be significant (p=0.500).

A) Interleukin-22



B) Interleukin-17A/F



C) Interleukin-22-RA2

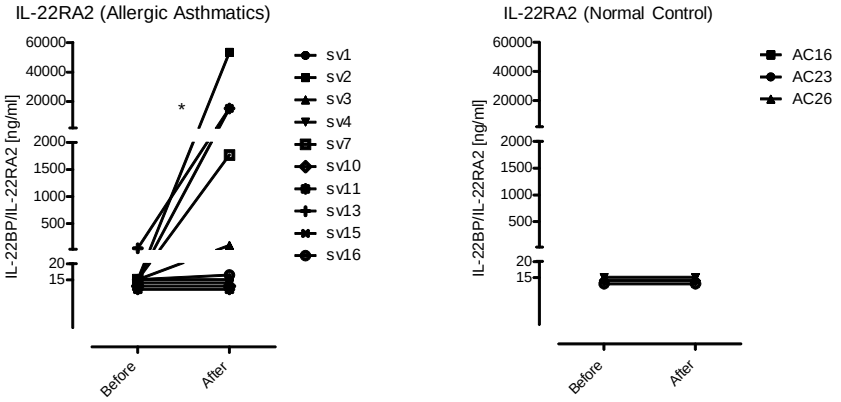


Figure 18. IL-22, IL-17A/F and IL-22RA2 ELISA measurements in (BAL) before and 24 hrs after segmental allergen challenge

Analysis: * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001 (Before vs. After: Wilcoxon; Allergic Asthmatic vs. Normals: Mann-Witney U-test)

IL-22 is commonly classified under a Th_{17} cell response and was thus measured together with IL-17A and IL-17F. In addition, a newly developed IL-22RA2 Sandwich ELISA (see Methods 1.1, page 72) was used to quantify the soluble IL-22 receptor before and after the allergen challenge.

The ELISA results showed that after allergen challenge IL-22 and IL-22RA2 increased in allergic asthmatics but not normal healthy controls (Figure 18). IL-22 increased in 8/15 asthmatics (p=0.008) from below the detection limit to 32.2(1.5) pg/ml. IL-22RA2 increased in 6/10 asthmatics (p=0.031) from less than 15 ng/ml to 5.74(0.03) µg/ml. IL-17A/F levels remained unchanged in both groups after allergen exposure.

1.2. Intradermal allergen challenge

The second study, a human intradermal allergen challenge to the skin (see Methods 1.2, page 74), allowed the study of tissue samples taken directly from the site of inflammation.

1.2.1. Demographics of study participants

Table 61. Clinical data of individuals who underwent intradermal grass pollen challenge and subsequent cutaneous punch biopsy

Treatment Group	Patients (n=14)	Controls (n=5)
Age (Y)	31.4(2.7)	30.2(2.9)
Gender (M/F)	9/5	4/1
Skin Prick Test-positive (Yes/No)	14/0	0/5
Multiple allergen (Yes/No)	8/6	0/5
Total IgE (IU/ml)	394.0(125.5)	16.4(4.1)
RAST (IU/ml)	46.2(9.6)	0.4(0.0)

Mean +/- standard error of mean (Std.Dev./√n)

14 atopic individuals with allergic rhinitis and 4 normal healthy controls were recruited (Table 61). Both groups were matched for their age and gender. Compared to normal healthy controls, atopic individuals had increased levels of total serum IgE ($p= 0.002$). In addition, atopic participants reacted positive to timothy grass by the skin prick test (>3mm) and had significantly elevated levels of timothy grass specific IgE in their bloodstream (RAST; $p= 0.001$).

1.2.2. Late phase skin response and cutaneous eosinophils

Late phase skin response

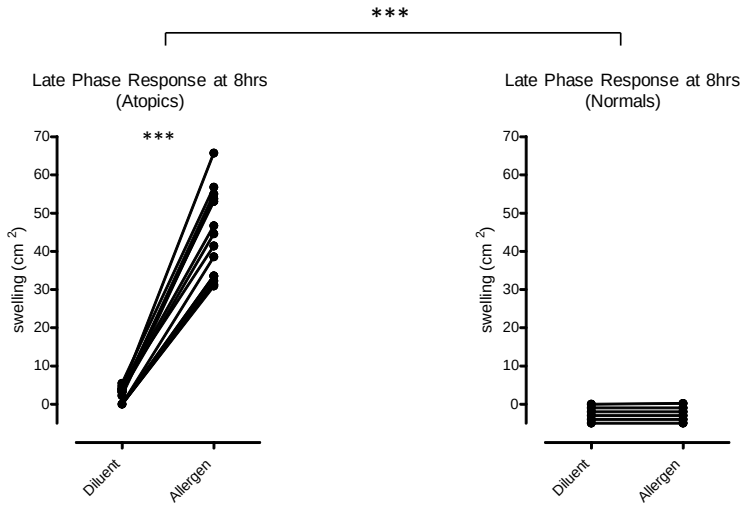


Figure 19. Measurement of the cutaneous swelling and reddening 8 hours after intradermal grass pollen challenge

Atopics (p=0.0005 Wilcoxon), Atopics vs Normals (p=0.0010 Mann Witney U test).

To assess a positive physical reaction towards the allergen, reddening and swelling of the skin was measured. Only atopic individuals showed a weal and flare reaction after 15 minutes (early response) and a delayed-in time swelling after 8 hours (late skin response). The measurements at 8 hours post challenge showed that allergen injection had caused significant swelling of 42.4(0.8) cm^2 compared to 2.2(0.6) cm^2 at baseline (p=0.001) (Figure 19).

Cutaneous eosinophils

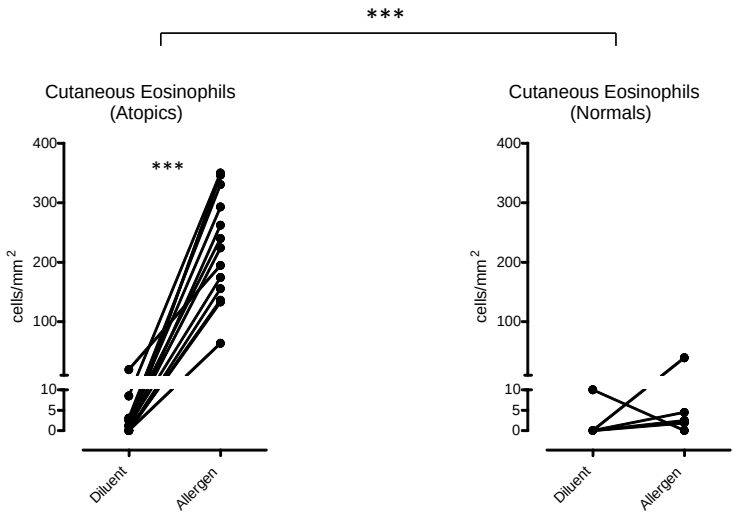


Figure 20. Quantification of cutaneous eosinophil (MBP-positive) cell numbers in tissue sections taken from individuals who underwent intradermal grass pollen challenge

Atopics (p=0.0005 Wilcoxon), Atopics vs Non-Atopics (p=0.0005 Mann Witney U test). Immunohistochemistry of MBP-positive cells (BMK13) Mean +/- standard error of mean.

To determine a positive allergic immune response in the biopsies taken from the site of allergen injection, tissue sections were stained against eosinophil-associated major basic protein (MBP) by immunohistochemistry and analysed by light microscopy.

The results of the immunohistochemical stain showed that MBP^+ cells increased at the site of allergen injection in 14/14 atopics and 3/4 normal healthy controls. In atopics MBP^+ cells increased 82-fold from 2.8(0.6) cells/mm^2 to 230.5(21.7) cells/mm^2 (p=0.001) (Figure 20). In normal healthy controls MBP^+ cells increased non-significantly from 1.6(1.6) to 8.0(6.3) cells/mm^2 (p=0.351).

1.2.3. IL-22 Immunofluorecent staining of skin sections

To test the hypothesis that T cells are the main source of IL-22 during the human allergic immune response, allergen challenged skin tissue was stained by immunohistochemistry and analysed by fluorescent microscopy (see Methods 1.2, page 76).

IL-22 vs T cells

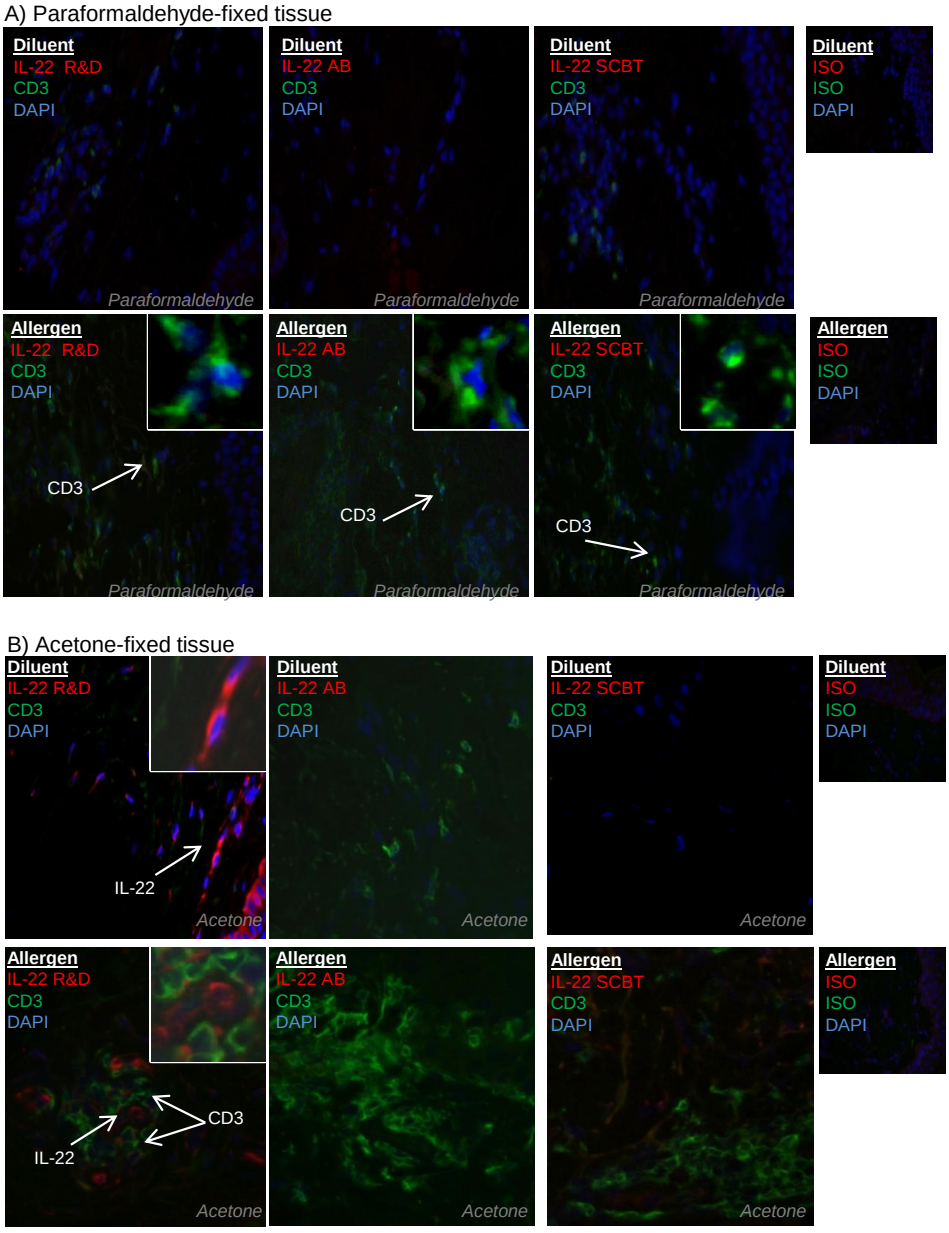


Figure 21. Immunofluorescent co-staining of IL-22 and CD3 in skin sections prepared from punch biopsies taken 8hrs post grass allergen-diluent or allergen challenge from grass allergic individuals.

A) Paraformadehyde-fixed tissue B) Acetone-fixed tissue

To identify IL-22, allergen challenge skin tissue was fixed in paraformaldehyde. Compared to Acetone fixation, this increases the likelihood of detecting a released mediator such as IL-22.

The results of the dual-immunofluorescent staining showed evidence of CD3 but not IL-22 expression in paraformaldehyde-fixed skin tissue (Figure 21A). None of the 3 previously validated IL-22 antibodies (see Methods 1.2, Figure 10, page 77) generated a visible signal in tissue taken either from the site of allergen or diluent injection. IL-22^{+} cells were only detected in acetone-fixed skin tissue by 1 of the 3 IL-22 antibodies (Figure 21B, IL-22 in red). This IL-22 antibody (R&D AF782) stained in both allergen and diluent challenged skin elongated and clustered as well as round and individually dispersed IL-22^{+} cells. On the other hand, CD3^{+} cells were only detectable after allergen injection and showed no immediate association with IL-22 (Figure 21, CD3 green).

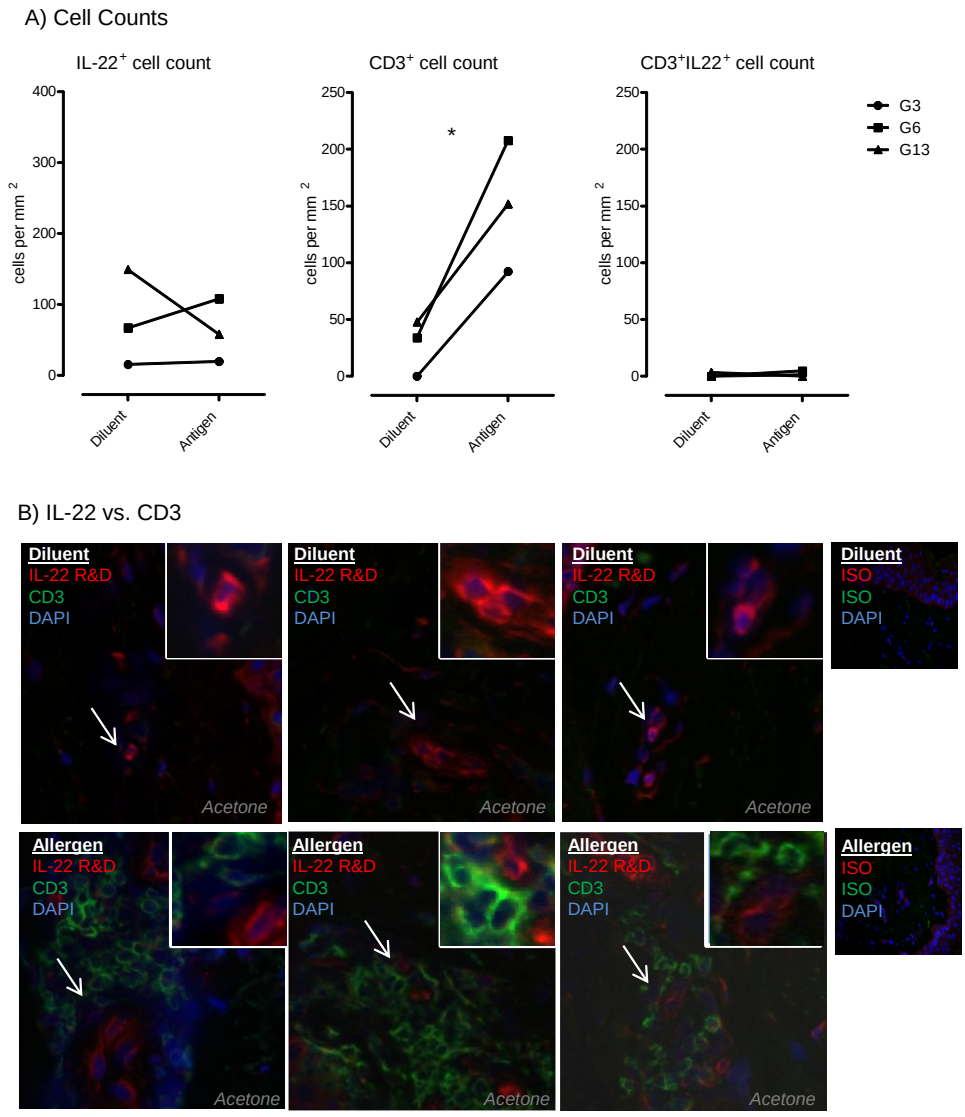


Figure 22. Immunofluorescent staining and quantification of CD3⁺ and IL-22⁺ cells in skin sections taken 8hrs post grass allergen challenge from 3 grass allergic individuals
A) Individual CD3, IL-22, and CD3/IL-22 cell counts ,increase in CD3 cell numbers (p= 0.0246 paired T-test) B) Immunofluorescent co-staining of 3 atopic patients with IL-22 and CD3 (from left to right G3,-6,-13)

Having identified a positive IL-22 signal, individual $CD3^+$, $IL\text{-}22^+$, as well as $CD3^+IL\text{-}22^+$ cell numbers were quantified using a checkerboard counting system in allergen challenged skin tissue taken from 3 atopic individuals (Figure 22).

The cell counting confirmed that $IL\text{-}22^+$ cells occurred both in allergen and diluent challenged skin across 3/3 atopic individuals (Figure 22A). $IL\text{-}22^+$ cells increased only minimally from 62.0(25.5) cells/mm^2 to 77.2(39.0) cells/mm^2 after allergen injection ($p>0.05$). $CD3^+$ cells, on the other hand, increased 5.5-fold from 27.3(14.2) to 150.6(33.3) cells/mm^2 ($p=0.02$). Double positive $CD3^+$ $IL\text{-}22^+$ cells were rare (<2 cells/mm^2) and remained unchanged after allergen injection.

IL-22 vs IL-22RA1

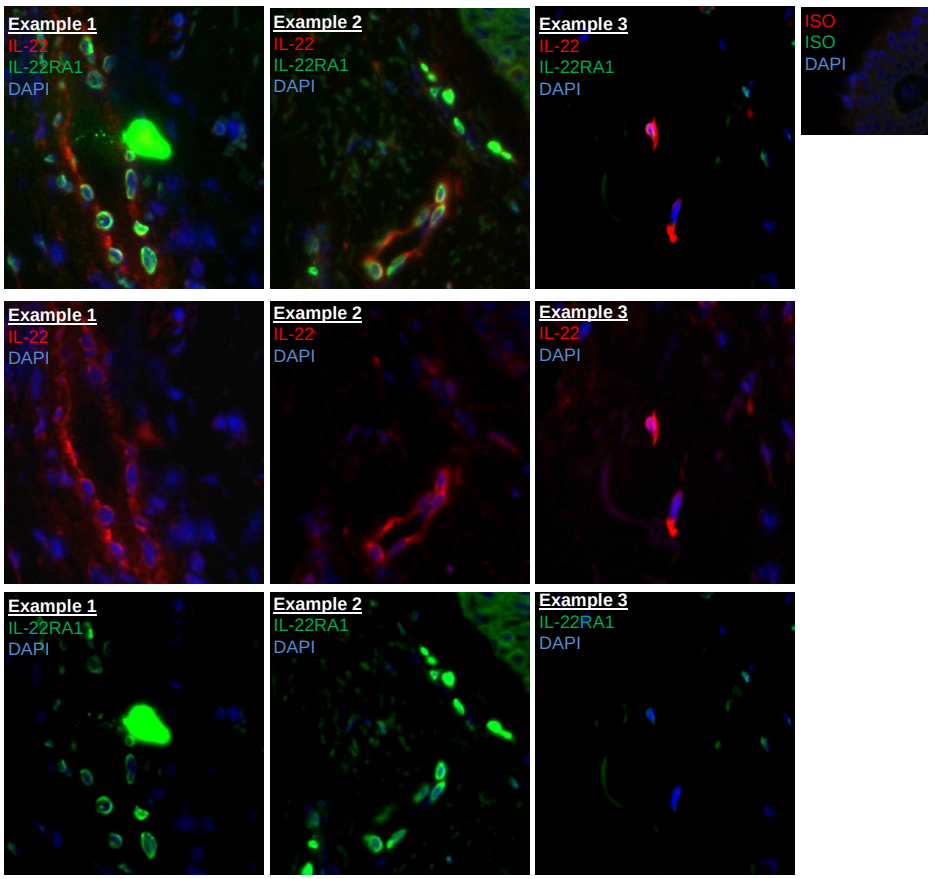


Figure 23. IL-22 Immunofluorescent co-staining to IL22 Receptor (RA1) in skin sections taken 8 hours after intradermal grass pollen challenge from grass allergic individuals
Shown are examples 1-3 were close proximity of IL-22 and IL-22RA1 staining occurred. For better visual examination each example is represented as a co-stain as well as individual single stain.

To discover if the observed IL-22 was receptor-bound, IL-22 was co-stained against IL-22RA1 (Figure 23). The results showed no overlap between IL-22 and IL-22RA1. The majority of IL-22^{+} (*red*) and IL-22RA1^{+} cells (*green*) were separate and morphologically distinct from each other.

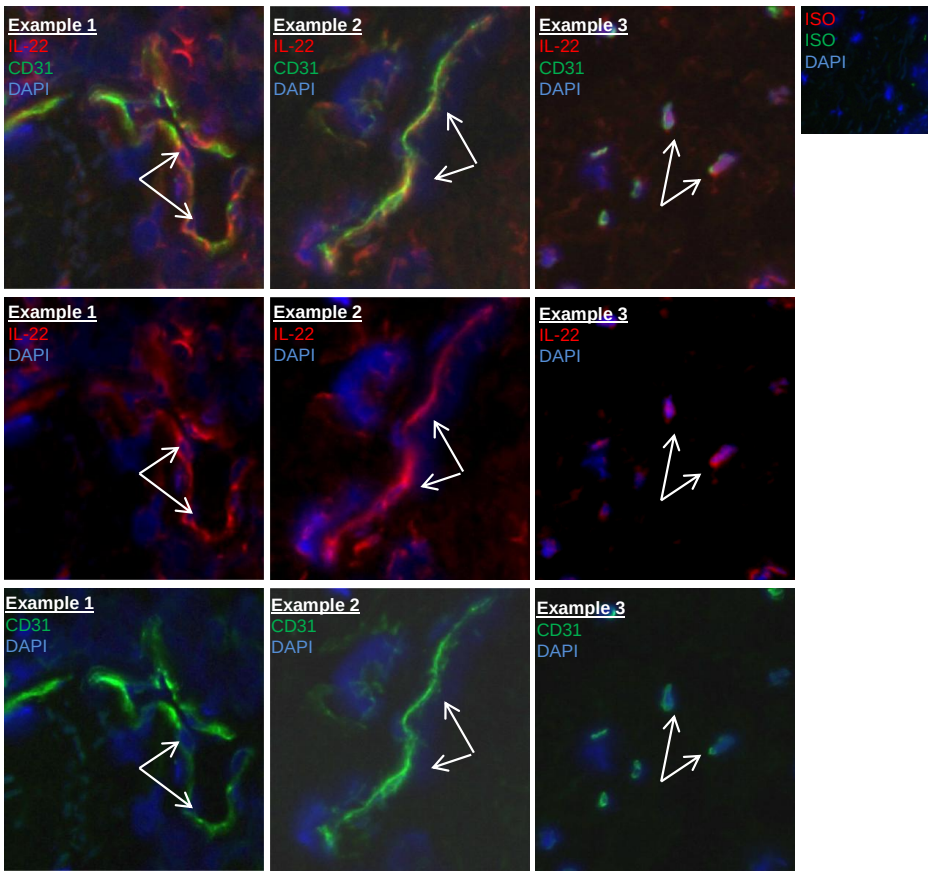


Figure 24. IL-22 Immunofluorescent co-staining to endothelial cells (CD31) in skin sections taken 8 hours after intradermal grass pollen challenge from grass allergic individuals

Shown are examples 1-3 were close proximity of IL-22 and CD31 staining occurred. For better visual examination each example is represented as a co-stain as well as individual single stain.

To understand if endothelial cells could account for the large and elongated IL-22 positive cells, IL-22 was co-stained against the endothelial marker CD31 (Figure 24). The result of the CD31/IL-22 co-stain showed that both stains strongly overlapped generating a yellow fluorescent signal. Not only long and elongated IL-22^{+} cells (Figure 24, Example 1-2) but also round and individually dispersed IL-22^{+} cells co-localised to CD31^{+} cells (Figure 24, Example 3).

1.2.4. IL-22 Receptor Immunofluorecent staining of skin sections

To understand the IL-22 receptor expression during allergic inflammation and discover the identity of IL-22 receptor positive cells, both IL-22RA1 and IL-22RA2 were investigated by immunohistochemistry and analysed by fluorescent microscopy (see Methods 1.2, page 76).

Overall IL-22RA1 and –RA2 expression

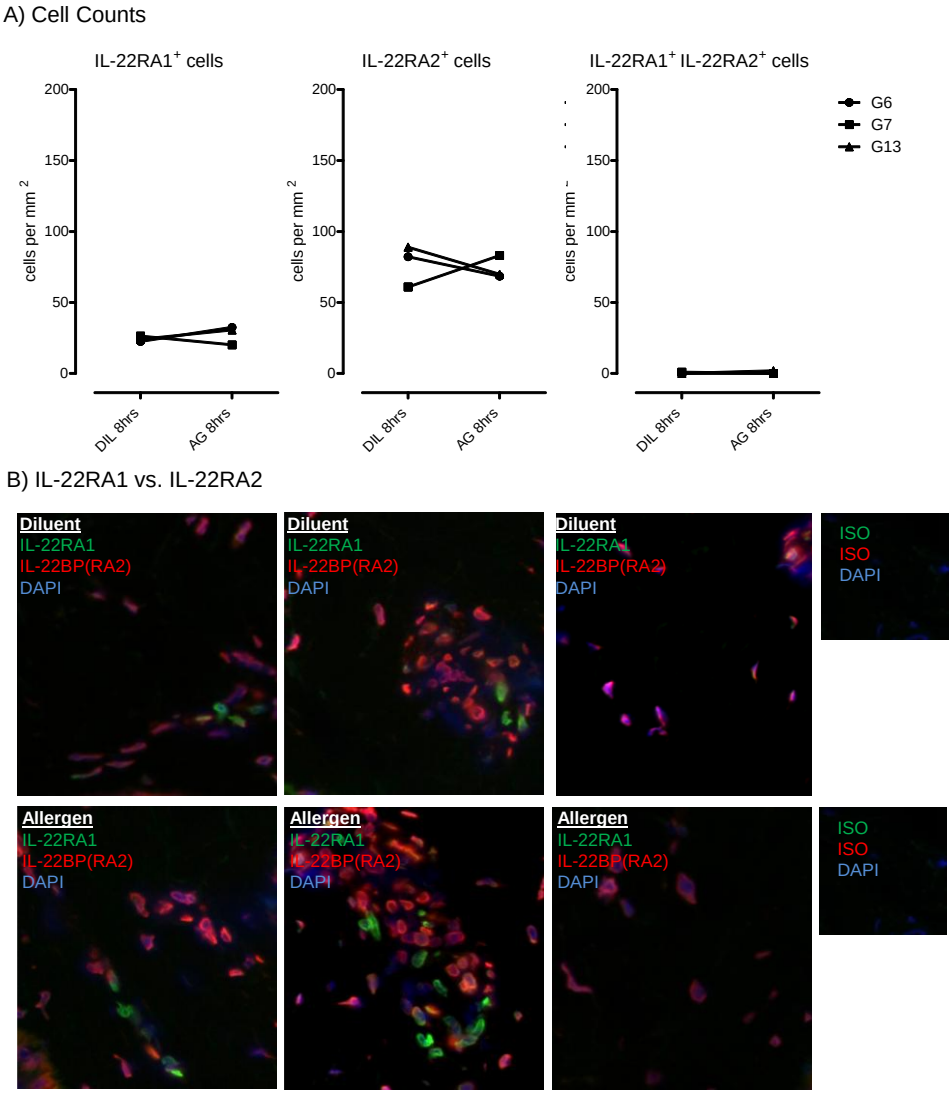


Figure 25. Immunofluorescent staining of IL-22 Receptor RA1 and RA2 positive cells in acetone-fixed skin sections taken 8 hours after intradermal grass pollen challenge from 3 grass allergic individuals

A) Individual IL-22RA1, RA2, and IL-22RA1/RA2 cell counts B) Immunofluorescent co-staining of 3 atopic patients with IL-22RA1 and RA2 (from left to right G6,-7,-13)

The results of the dual-immunofluorescence staining showed that IL-22RA1$^+$ and -RA2$^+$ cells occurred in both allergen and diluent challenged skin and remained unchanged during allergic inflammation (Figure 25). 3 times more IL-22RA2$^+$ (77.4[8.4] cells/mm^2) than IL-22RA1$^+$ (27.8[3.8] cells/mm^2) were identified in allergen and diluent challenged skin (Figure 25A).

Expression in immune cells

To understand if resident and infiltrating immune cells expressed IL-22RA1 or -RA2, both receptors were co-stained against adaptive and innate immune cell markers and assessed by fluorescent microscopy.

IL-22RA1 and –RA2 vs adaptive immune cells

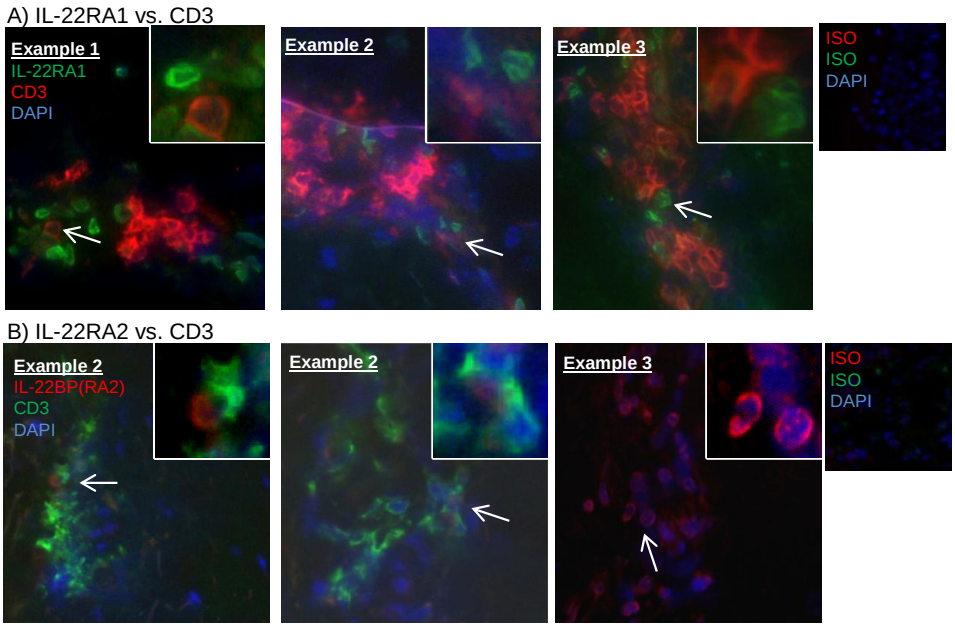


Figure 26. IL-22 Receptor (RA1/RA2) Immunofluorescent co-staining to T cells (CD3) in acetone-fixed skin sections taken 8 hours after intradermal grass pollen challenge from grass allergic individuals

A) IL-22RA1 and CD3 co-stain B) IL-22RA2 and CD3 co-stain. Represented are 3 examples were both Receptor and CD3 staining occurred in close proximity. The tissue was fixed in acetone.

First, infiltrating T (CD3) and B cells (CD19) were investigated for IL-22RA1, and –RA2 expression. The dual-immunofluorescent stain showed no evidence that infiltrating $CD3^+$ cells expressed IL-22RA1 or -RA2 (Figure 26). $CD3^+$ cells were morphologically distinct and approximately 1.2-1.5 times smaller than $IL\text{-}22RA1^+$ and $RA2^+$ cells. While $CD3^+$ cells were readily identified, $CD19^+$ cells were absent in tissue obtained 8 hours after allergen injection (data not shown).

IL-22RA2 vs innate immune cells

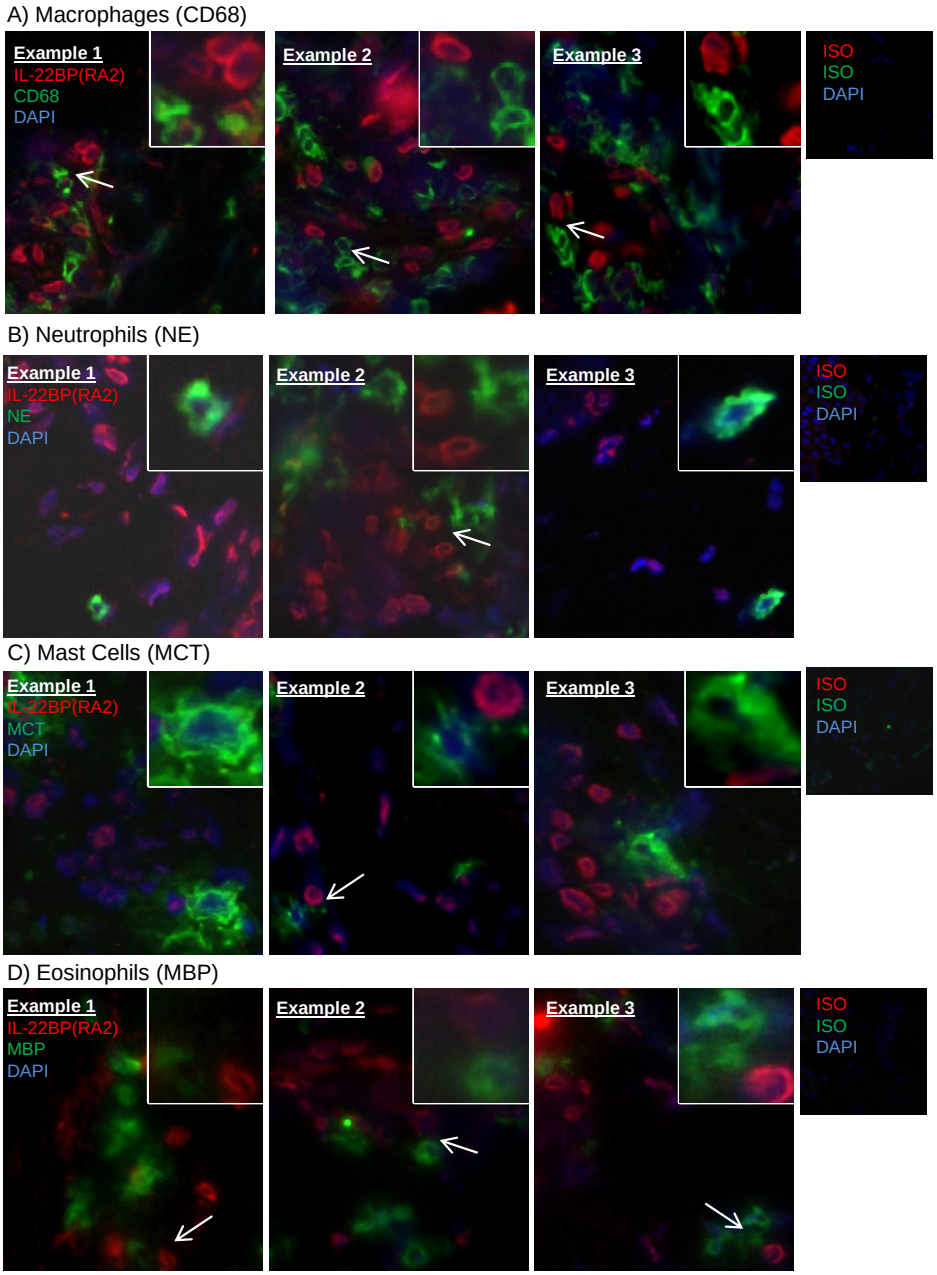


Figure 27. IL-22 Receptor RA2 co-staining to innate immune cells in acetone-fixed skin sections taken 8 hours after intradermal grass pollen challenge from grass allergic individuals

IL-22RA2 was co-stained to A) CD68 B) Neutrophil elastase C) Mast cell tryptase D) Major basic Protein. Represented are 3 examples were both IL22 Receptor 2 and the respective co-stain occurred in close proximity. The tissue was fixed in acetone.

IL-22RA2 was co-stained against the cellular markers of macrophages/dendritic cells (CD68), neutrophils (neutrophil elastase), mast cells (mast cell tryptase), and eosinophils (major basic protein).

Each of the cell populations was readily identified within the allergen challenged skin tissue (Figure 27). IL-22RA2 did neither co-localise to macrophages, neutrophils, mast cells, nor eosinophils (Figure 27A-D).

Expression in structural cells

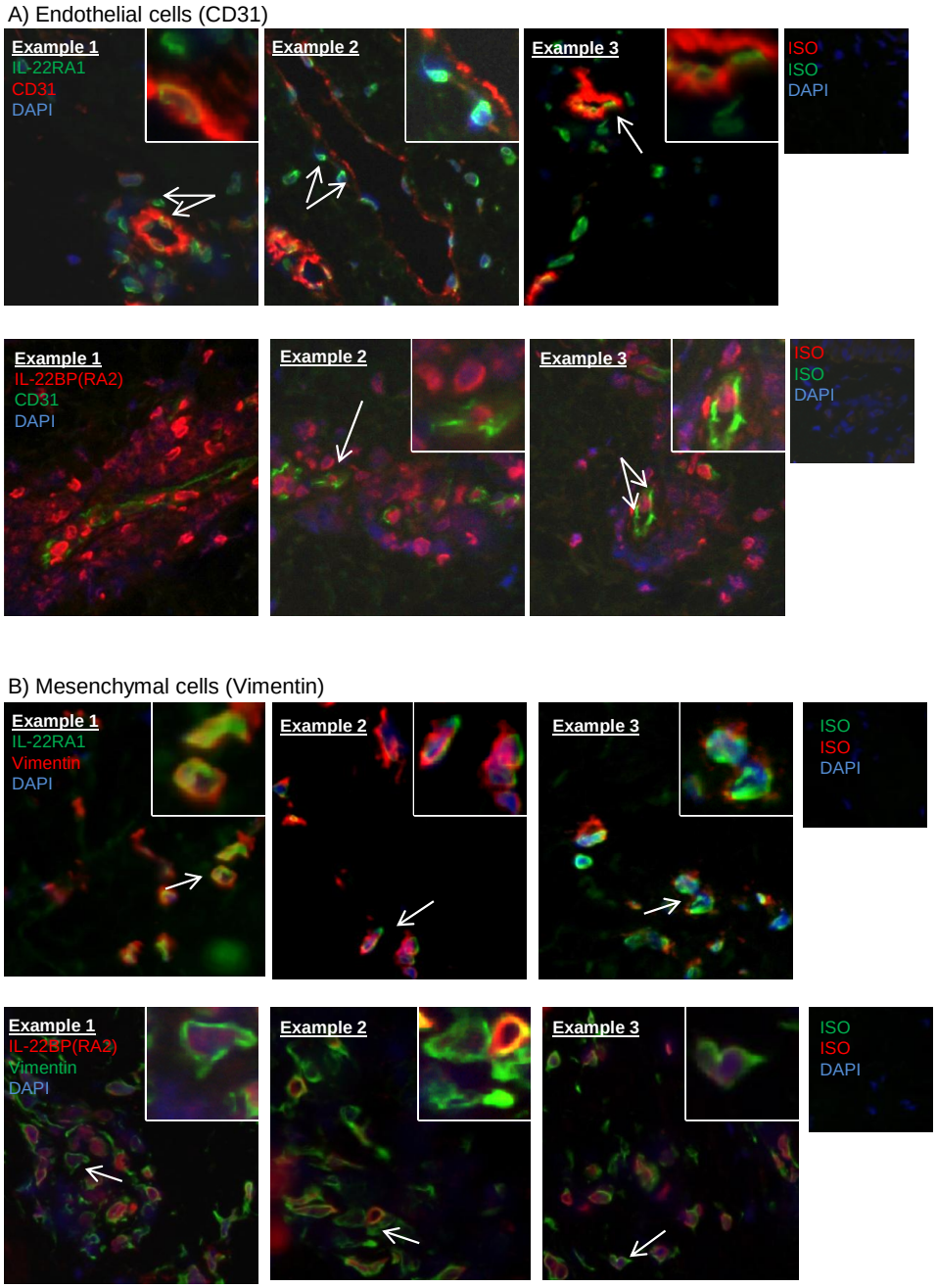


Figure 28. IL-22 Receptor RA1/RA2 co-staining to endothelial (CD31) and mesenchymal (Vimentin) cells in acetone-fixed skin sections taken 8 hours after intradermal grass pollen challenge from 3 grass allergic individuals

A) IL-22RA1 and RA2 co-staining against CD31 B) IL-22RA1 and RA2 co-staining against Vimentin

To understand if IL-22RA1 and –RA2 could be expressed by structural cells, an analysis of their expression in the epidermis, blood vessels and connective tissue was carried out. The analysis of the epidermis showed that it stained positive for both IL-22RA1 and –RA2. This stain remained inconclusive as the epidermis was stained by both isotype control antibodies (data not shown).

To identify the expression of IL-22RA1 and –RA2 on blood vessels, the endothelial cell marker CD31 was used. The results showed that neither IL-22RA1^{+} or –RA2^{+} cells co-localised to CD31 (Figure 28A).

Cells of the connective tissue were identified using the mesenchymal cell marker vimentin. The results showed that both IL-22RA1^{+} and -RA2^{+} cells almost exclusively co-localised to vimentin^{+} cells (Figure 28B).

IL-22RA2 in Fibroblasts

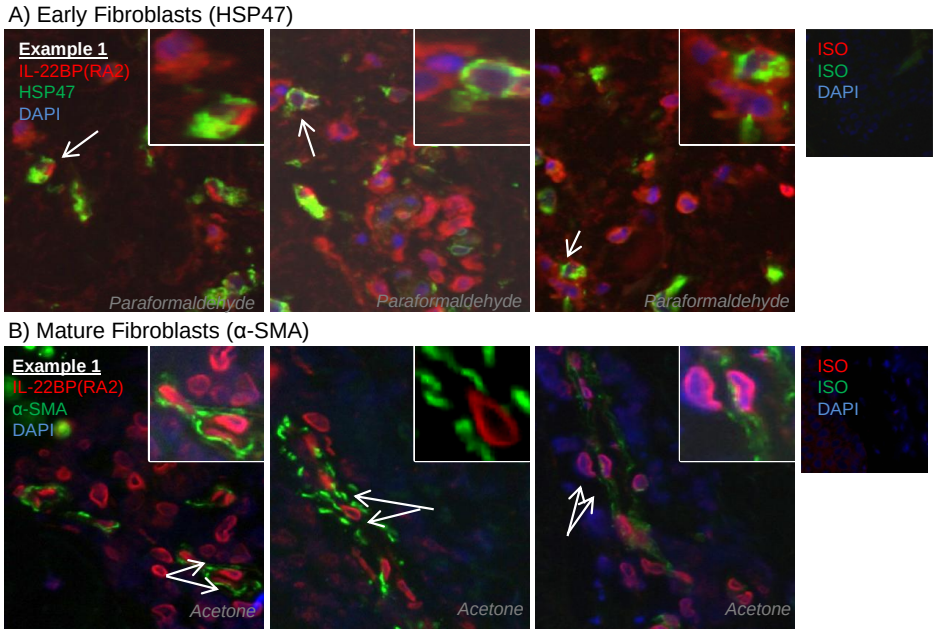


Figure 29. IL-22 Receptor RA2 co-staining to fibroblasts in skin sections taken 8 hours after intradermal grass pollen challenge from 3 grass allergic individuals

A) IL-22RA2 and Heat shock protein 47 co-stain (Paraformaldehyde fixed tissue) B) IL-22RA2 and alpha-Smooth-Muscle-Actin co-stain (acetone fixed tissue).

A major constituent of the connective tissue are fibroblasts. To investigate if fibroblasts accounted partially for the vimentin$^+$ stain, IL-22RA2 was co-stained against an early and late stage marker of fibroblasts.

To identify the early stage fibroblasts, Heat-shock-protein-47 (HSP47), a chaperone protein for collagen was stained against. The results showed that HSP47$^+$ cells were almost always co-stained with IL-22RA2 (Figure 29A) but could not account for all IL-22RA2$^+$ cells. A substantial number of IL-22RA2$^+$ cells were HSP47 negative.

To identify late stage fibroblasts, alpha-smooth-muscle-actin (α-SMA), which forms an integral part of the contractive apparatus of mature fibroblasts (Arora *et al* 1994) was stained against. Co-staining of IL-22RA2 against α-SMA showed no obvious association between the two (Figure 29B). The morphology of α-SMA$^+$ cells was very distinct from IL-22RA2$^+$ cells. α-SMA$^+$ cells appeared in clusters and as thin and highly elongated cells while IL-22RA2 $^+$ cells were round and individually dispersed.

1.3. Summary

Bronchial segmental allergen challenge

- Bronchial segmental allergen challenge caused a significant immediate reduction in lung function (FEV_1) in allergic asthmatics but not normal controls
- Bronchoalveolar lavage of allergic asthmatics taken 24 hours after allergen challenge showed a significant influx of eosinophils and IL-5 but not IFNγ expression
- Measurement of IL-22, IL-22RA2 and IL-17 showed that IL-22 and IL-22RA2 but not IL-17A/F significantly increased after allergen challenge

Intradermal allergen challenge

- Intradermal allergen challenge caused both an early and significant late phase cutaneous swelling in atopic allergic subjects but not normal controls
- Tissue biopsies taken from the site of allergen inflammation showed a significant increase in eosinophils and T cells 8 hours after challenge
- Detection of IL-22 remained inconclusive and could not be convincingly assessed using Immunfluorescence
- IL-22RA1 and RA2 receptor expression showed a wide constitutive expression pattern within the skin which remained unchanged after allergen challenge
- Both IL-22RA1$^+$ and RA2$^+$ were largely confined to the vimentin$^+$ cells
- Co-localistaion of IL-22RA2 showing some co-expression with HSP47 but not αSMA

2. IL-22 expression in T cells

We hypothesised that T cells were the main source of IL-22 during the allergic inflammation. As we were unable to stain IL-22 in tissue taken from the site of allergen inflammation (see Results 1.2.3, page 108), we set out to test our hypothesis *in vitro*.

2.1. from peripheral blood

Using peripheral blood, an *in vitro* allergen challenge model was set up to measure the induction of IL-22 and IL-22$^+$ T cells. In addition the study aimed to investigate IL-22$^+$ T cells more generally. To do so naïve T cells were polarized in newly described Th_{22} differentiating conditions and the expression of putative Th_{22} receptors in memory T cells was evaluated.

2.1.1. long term allergen PBMC culture

To study the induction of IL-22 and IL-22$^+$ T cells as a response to allergen, an *in vitro* challenge model, in which peripheral blood mononuclear cells (PBMCs) were stimulated with purified allergen over a period of 6 days, was set up. (see Methods 2.1.1, page 79).

Demographics of study participants

Table 62. Summary of the clinical parameters of individuals whose blood was taken and used for the in-vitro allergen PBMC co-culture experiments

Treatment Group	Atopic (n=12)	Normal Control (n=11)
Age (Y)	33.2(2.5)	45.4(4.2)
Gender (M/F)	5/7	7/4
Skin prick test positive (Yes/No)	12/0	0/12
Multiple allergen (Yes/No)	9/3	0/12
Total IgE (IU/ml)	290.5(82.9) **	28.8(9.2)
RAST (IU/ml)	24.3(7.1) ****	0.01(0.0)

Skin Prick test against Timothy Grass (*Phleum pretanse*). *Total IgE and RAST for one normal control (Tc209) were not available; Mean +/- Standard Error of the Mean (Std.Dev./√n). Statistical test (Mann-Witney U test)

In total, 12 atopic individuals with seasonal allergic rhinitis and 11 normal healthy control individuals were recruited (Table 62). Both groups had a similar distribution of age and gender. Compared to normal healthy controls, atopic individuals had significantly higher levels of total serum IgE (p=0.003). All atopic patients tested positive by skin prick testing to timothy grass (*Phleum pratense*) and had significantly elevated levels of specific IgE in their serum (RAST; p= 0.0001).

[methyl^{3}H]-Thymidine incorporation

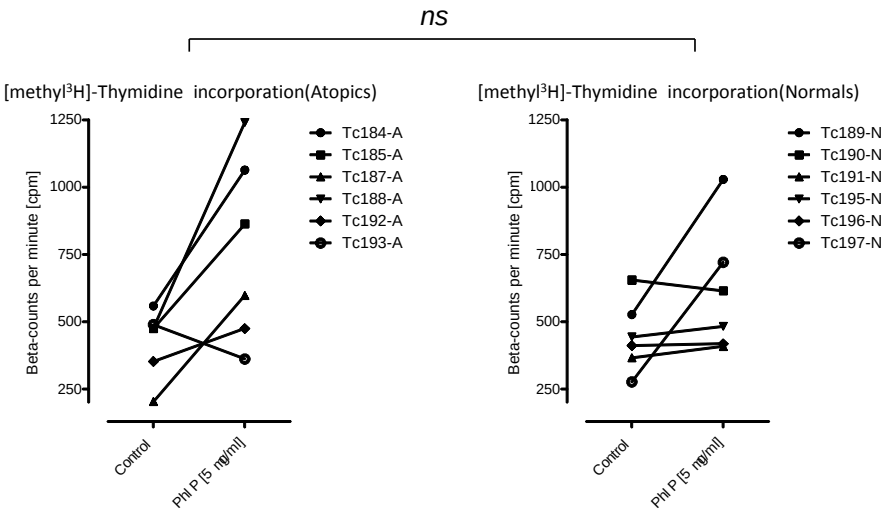


Figure 30. Measurement of [methyl^{3}H]-Thymidine incorporation over 16 hours in PBMCs obtained after 6 days of allergen-PMBC co-culture from grass pollen atopic and normal individuals

To determine a positive proliferative response of PBMCs to *Phleum pratense* [methyl^{3}H]-thymidine was added in a subset of participants to their culture and its incorporation quantified (see Methods 2.1.1, page 79). The results of the [methyl^{3}H]--thymidine incorporation showed that cultures derived from atopic donors showed an 80% increase in [methyl^{3}H]-thymidine incorporation from 426.1(4.6)cpm to 767.2(7.6)cpm after allergen challenge (Figure 30). This increase was convincing in 5/6 atopics but due to one outlier failed to reach significance (p=0.09).

The [methyl^{3}H]-thymidine incorporation results of cultures derived from normal donors showed a more mild increase in [methyl^{3}H]-thymidine absorption of 37%. This increase was found in 5/6 normal donors. The difference between atopic and normal PBMC cultures in the proliferative response towards allergen was visible but not significant (p=0.394).

Cytokine expression in culture supernatants

IL-5 and IFNγ

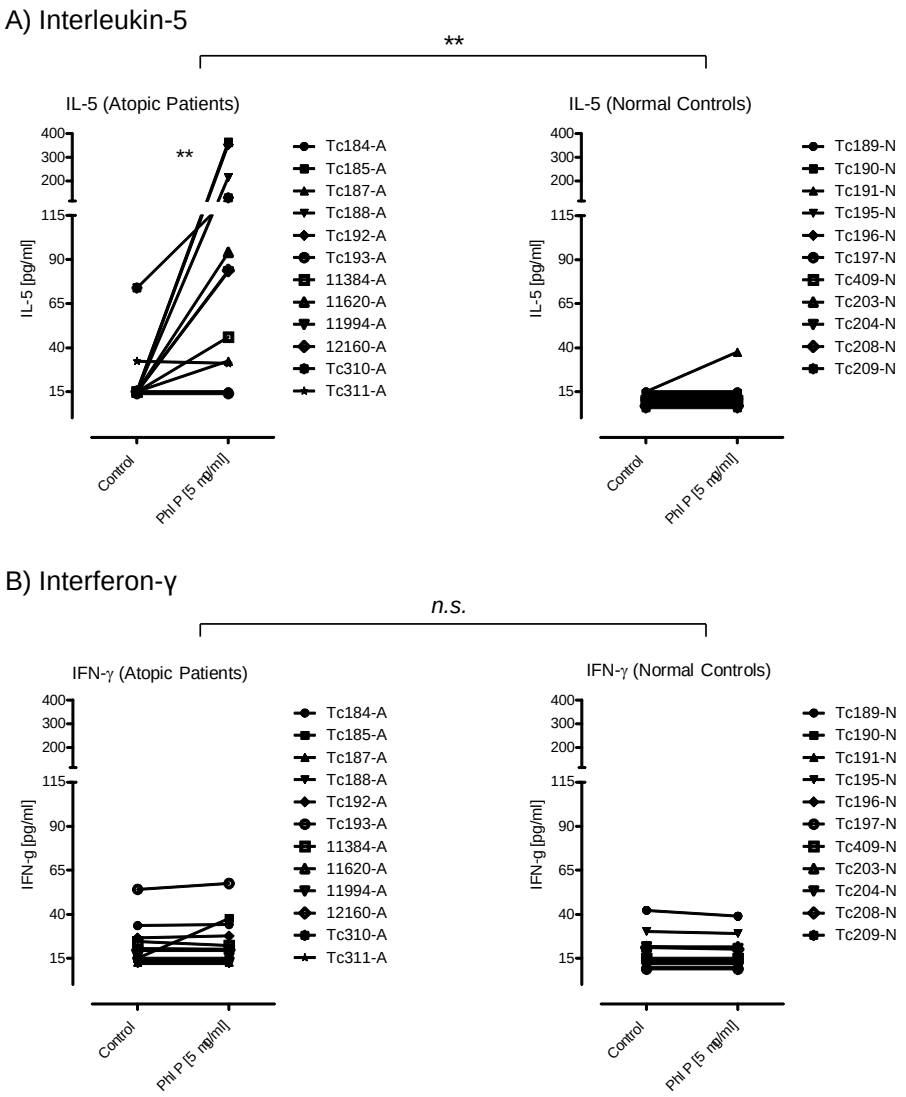


Figure 31. Quantification of IL-5 and IFNγ in culture supernatants obtained from 6 day allergen-PMBC cultures of both grass allergic and normal donors.

A) Change in IL5 Atopics p=0.0039 Wilcoxon. Change in IL-5 Atopics vs Normals p=0.0042 Mann-Witney U test.

To determine that *Phleum pratense* stimulation caused a Th_2 -type immune response in the PBMC cultures, the expression of the Th_2 cytokine Interleukin-5 and the Th_1 cytokine IFN-γ within culture supernantants were quantified.

The measurement of IL-5 by ELISA showed that the cytokine increased in 8/12 atopic (p=0.004) PBMC cultures after allergen stimulation (Figure 31A). This observation was significantly different to the normal PBMC cultures (p=0.004) where only 1/11 had detectable levels of IL-5 after allergen stimulation. The measurement of IFN-γ showed that the cytokine

remained unchanged after allergen stimulation in both atopic and normal PBMC cultures (Figure 31B).

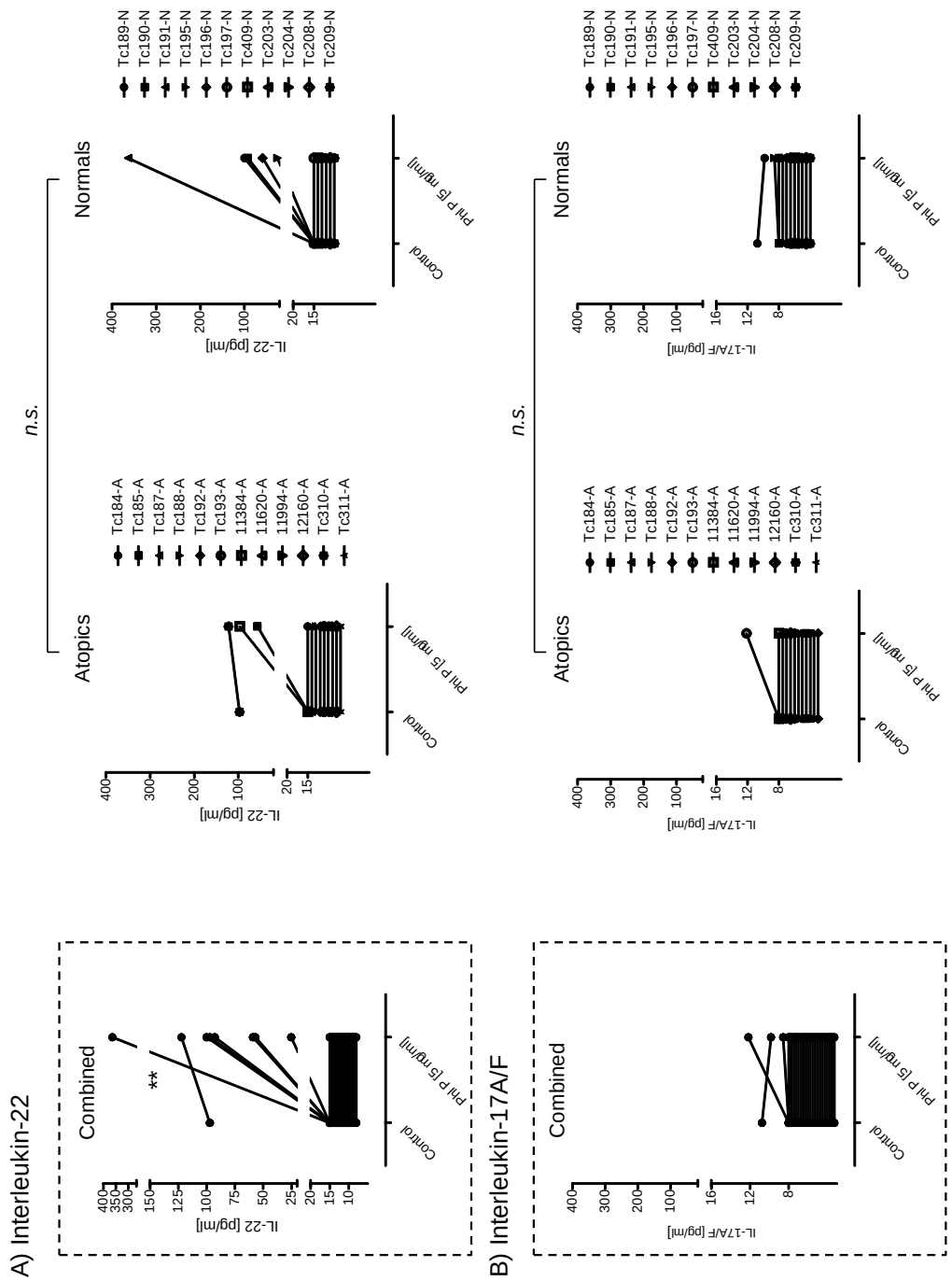


Figure 32. Quantification of IL-22 and IL-17A/F in culture supernatants obtained from 6 day allergen-PMBC cultures of both grass allergic and normal donors.

Statistaical analysis (p=0.004; Wilcoxon matched paired T test)

IL-22 is commonly classified under a Th_{17} cell response and was thus quantified together with IL-17A and IL-17F by ELISA. The combination of both atopic and normal PBMC culture results (n=23) showed that IL-22 was significantly up-regulated after allergen challenge regardless of atopic status (p=0.004).The results showed that IL-22 increased in 3/12 atopic and 5/11 normal PBMC cultures after allergen stimulation (p>0.05) (Figure 32A). A power analysis showed that the additional recruitment of 37 atopics and 28 normal healthy controls may have achieved statistical significance in each group (p<0.05 Wilcoxon). The results of the IL-17A/F ELISA showed no evidence that IL-17 was elevated after allergen stimulation in either atopics or normal healthy controls (Figure 32B).

Flow cytometry analysis of IL-22$^+$ CD4$^+$ T cells

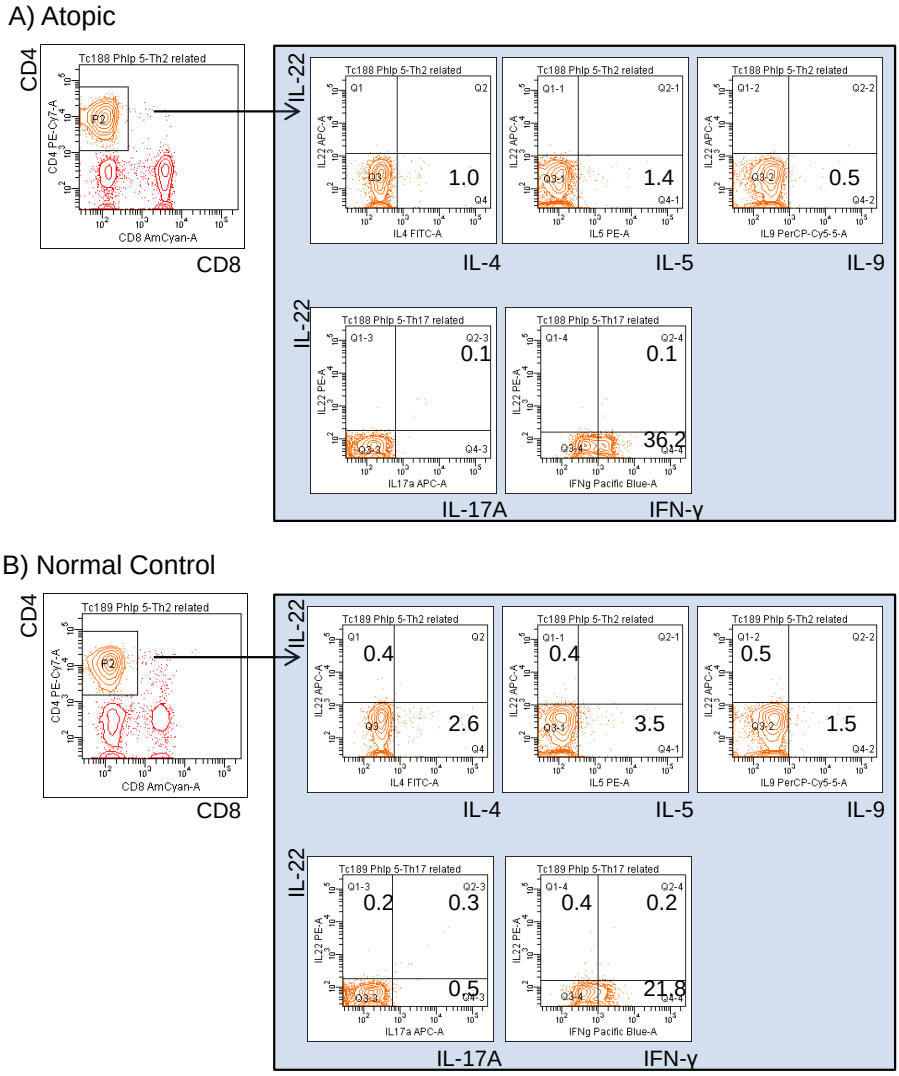


Figure 33. Flow cytometric analysis of IL-22 expression in CD4$^+$ T cells obtained after 6 days of grass allergen PBMC co-culture and subsequent PMA/Ionomycin stimulation.

A) Atopic allergen-PBMC culture (donor Tc188) B) Normal allergen-PBMC co-culture (donor Tc189). FACS plots are representative

To investigate if the allergen-induced IL-22 was released by T cells, the cultures were analysed further using flow cytometry (see method 2.1.1, page 80). At day 6, the allergen challenged PBMC cultures were polyclonally activated and stained for IL-22, IL-17A, IFN-γ, IL-4, IL-5, and IL-9 expression.

The flow cytometry results showed only traces of IL-22 expression in 0/12 atopic and 2/11 normal PBMC cultures (Figure 33). These small amounts of IL-22 were located exclusively in the CD4$^+$ T cell population. In the rare instance where IL-22 was detected, it did not co-

localise to the Th_2 cytokines IL-4, IL-5 or IL-9 but partially overlapped with The Th_1 and Th_{17} cytokines IFNγ and IL-17A.

2.1.2. polarised naive T cells

To study IL-22$^+$ T cells and their relationship to other T helper subsets in more detail, a Th_{22} differentiation culture using naive CD4$^+$ T cells was set up (see methods 2.1.2, page 81).

IL-22 expression after Th_{22} differentiation

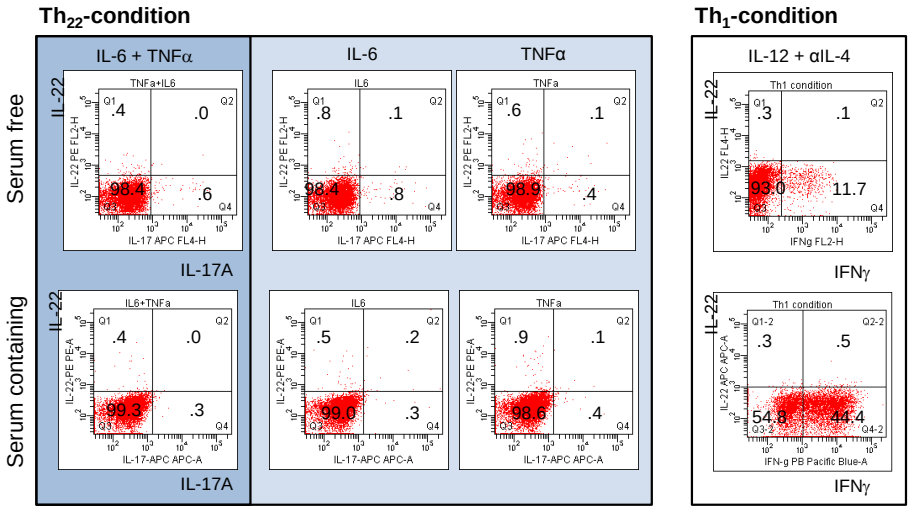


Figure 34. Flow cytometric analysis of IL-22 and IL-17A expression in T cells obtained after naive (CD45RO$^-$) peripheral blood T cell differentiation using Th_{22} and Th_1 deforming conditions.

FACS plots are representative (sample size n>3 per condition)

The newly described Th_{22} conditions of IL-6 and TNFα were used to deform naive T cells over a period of two weeks (Duhen *et al* 2009). After the differentiation, each T cell culture was polyclonally activated and analysed by flow cytometry for their IL-22 and IL-17A expression.

The results showed that IL-22 expression was low (<1%) and remained unchanged in cells exposed either to IL-6+TNFα, IL-6, or TNFα alone (Figure 34). These findings were identical when the serum free media (AIM-V) was replaced with serum containing media (RPMI+5% human serum). There was a tendency of IL-17A to be increased in "IL-6 only" cultures but this was not significant (p=0.3).

Along with Th_{22} polarising conditions, Th_1 polarising conditions were used as a positive control. The differentiation of naive T cells in IL-12 and α-IL-4 conditions showed to significantly increase IFNγ positive T cells. In serum free media, IFNγ expression increased

4.5-fold from 2.6(0.3)% to 11.9(2.3)% of total T cells (p=0.041). In serum containing media, IFNγ was further up regulated 7.5-fold from 5.6(0.6)% to 41.8(1.9)% (p=0.002). While the Th_1 polarising conditions increased IFNγ expression, IL-22 expression remained unchanged.

2.1.3. memory T cells

In addition to studying the development of IL-22^+ T cells, the study focused on ways to isolate and study already differentiated IL-22^+ memory T cells *ex vivo* (see Methods 2.1.3, page 84).

Occurrence of putative Th_{22} receptors FGFR2, PDGFR, TIE1 on T cells

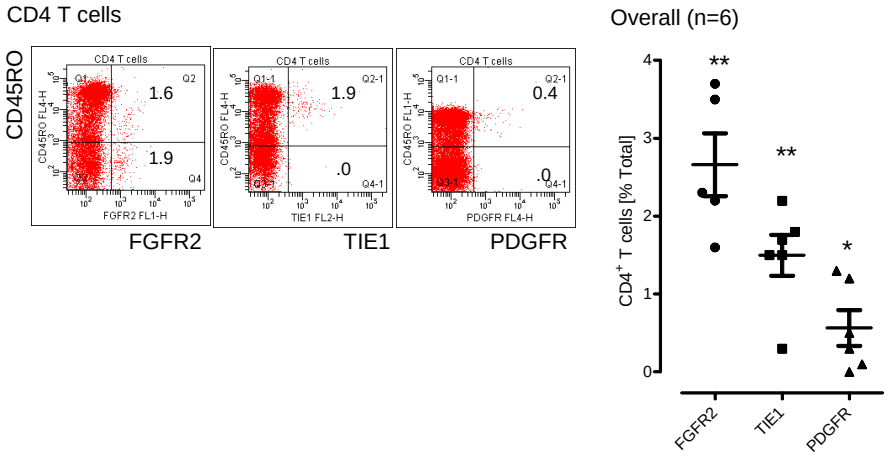


Figure 35. Flow cytometric analysis of putative Th_{22} receptor FGFR2, TIE1, PDGFR expression on peripheral blood $CD4^+$ T cells

FACS plots are representative. Statistical analysis of cumulative data (Receptor $^+$ vs. Isotype$^+$; paired T-test)

Populations of memory $CD4^+$ T cells have been shown to preferentially express specific surface receptors which can be used to enrich them from the peripheral blood (O'Garra *et al* 1998). The recent investigation of skin Th_{22} clones (Eyerich *et al* 2009) identified three surface receptors which may be preferentially expressed by IL-22 expressing $CD4^+$ T cells. These included the fibroblast-growth-factor-receptor-2 (FGFR2), the tyrosine-kinase-with-immunoglobulin-like-and-EGF-like-domain-receptor-1 (TIE1), and the platelet-derived-growth-factor-receptor (PDGFR).

First, it was established if FGFR2, TIE1, and PDGFR could be identified on peripheral blood $CD4^+$ T cells. The flow cytometry results showed that all 3 receptors were present on a small proportion of $CD4^+$ T cells (Figure 35). FGFR expression was found on 2.6(0.4)% of total $CD4^+$ T cells (p=0.003 vs isotype). Its expression was found equally on $CD45RO^+$ and $CD45RO^-$ $CD4^+$ T cells. TIE1 was expressed by 1.5(0.2)% of total $CD4^+$ T cells (p=0.002 vs isotype) all of which were exclusively $CD45RO^+$. PDGFR expression was barely detectable

and found on only 0.6(0.2)% of total $CD4^+$ T cells (p=0.043 vs isotype). Like TIE1, PDGFR expression was restricted exclusively to $CD45RO^+$ $CD4^+$ T cells.

IL-22 expression in putative Th_{22} receptor enriched cells

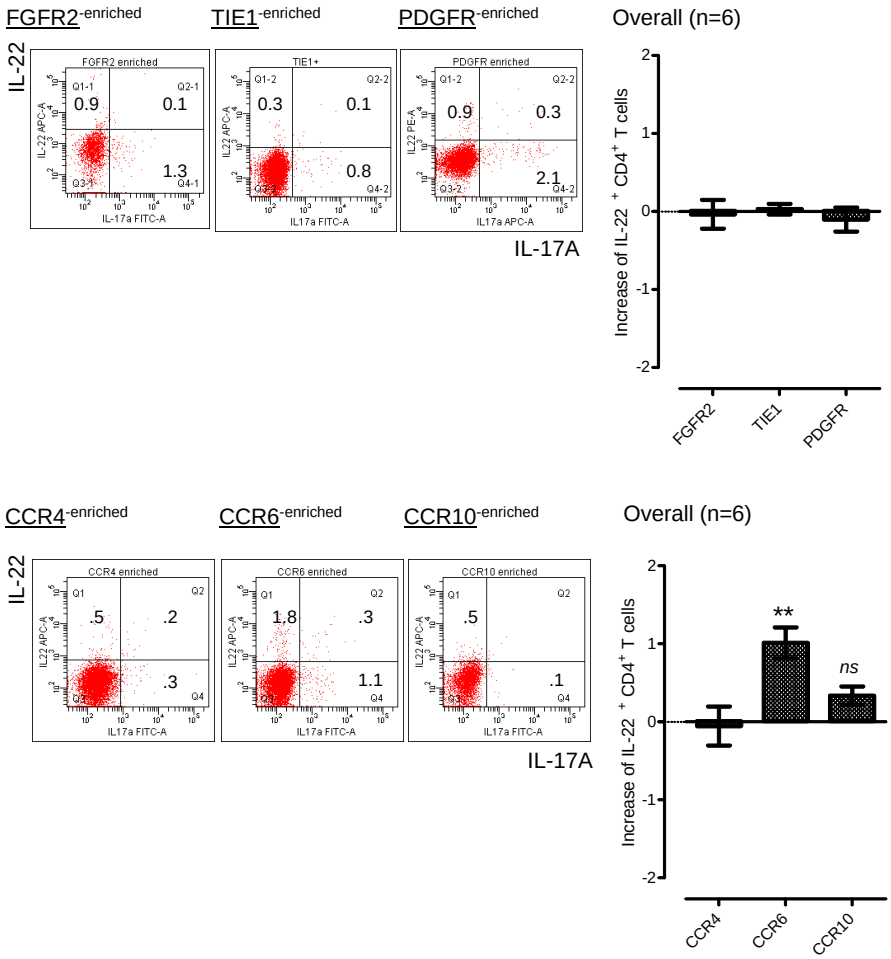


Figure 36. Flow cytometric analysis of IL-22 and IL-17A expression in magnetic-bead enriched peripheral blood T cells expressing putative Th_{22} receptors PDGFR, FGFR2, TIE1 or chemokine receptor CCR4, CCR6, CCR10.

FACS plots are representative. Statistical analysis of cumulative data (Receptor-enriched vs, Receptor-depleted $IL\text{-}22^+$ cells; paired T test)

To understand if FGFR2, TIE, or PDGFR positive T cells were linked to IL-22 expression, peripheral blood T cells were enriched using a combination of anti-receptor FACS antibodies and anti-fluorochrome magnetic beads (see Methods 2.1.3, page 84).

The resulting T cell fractions were polyclonally activated and analysed for their IL-22 and IL-17A expression by flow cytometry. The results showed that FGFR2, TIE1, and PDGFR enriched T cell fractions did not express more IL-22 than their respective depleted fractions (Figure 36). This was true not only for IL-22 but also IL-17A (data not shown). To verify that

IL-22$^+$ T cells could be enriched from peripheral blood CD4$^+$ T cells, we utilised the Th_{22}-associated chemokine receptors CCR4, CCR6, and CCR10 (Duhen *et al* 2009, Trifani and Spits 2010). The results of the enriched CCR$^+$ T cell fractions showed that IL-22 expression was increased minimally in CCR10-enriched and significantly in CCR6-enriched CD4$^+$ T cells (p=0.003).

IL-22 expression in putative Th_{22} receptor-ligand stimulated cells

While FGFR2-, TIE1-, and PDGFR-enriched CD4$^+$ T cells did not express more IL-22 than their depleted counterparts, it was investigated if the receptor ligands could trigger IL-22 production. To investigate this, peripheral blood CD4$^+$ T cells were cultured in the presence or absence of FGF-1, -2,-5 and PDGF-BB and their cytokine gene expression profile was analysed by RT-PCR (see Methods 2.1.3, page 85)

Table 63. Effect of 24 hour FGF-1,-2,-5 and PDGF-BB exposure on T cell cytokine mRNA expression of cultured peripheral blood CD4$^+$ T cells

Ligand	IL-22	IL-17A	IL-4	IFNγ	IL-10	TGFβ1	Effect
FGF-1	0.8(0.1)	0.9(0.1)	0.7(0.1)	0.8(0.2)	0.7(0.2)	0.9(0.2)	*No effect*
FGF-2	0.7(0.1)	1.0(0.6)	0.7(0.1)	0.8(0.1)	0.9(0.1)	1.1(0.2)	*No effect*
FGF5	0.8(0.1)	1.5(0.7)	1.1(0.3)	1.1(0.2)	1.1(0.3)	1.3(0.3)	*No effect*
PDGF-BB	0.8(0.1)	0.7(0.4)	0.7(0.1)	1.0(0.1)	1.0(0.1)	1.1(0.2)	*No effect*

Gene expression relative to un-stimulated cells and calculated using ΔΔ ct method. Data is represented as Mean +/- Standard Error of the Mean (Std.Dev./√n)

The results showed that the addition of FGF-1, -2, -5 and PDGF-BB for a duration of 24 hours did not alter the relative expression levels of IL-22, IL-17A, IL-4, IFNγ, IL-10, or TGFβ1 (Table 63).

2.2. from human asthmatic bronchial mucosa

To study lung memory IL-22$^+$ T cells in more detail, T cells were isolated directly from human asthmatic bronchial mucosa.

2.2.1. Demographics of study participants

Table 64. Summary of the clinical parameters of individuals who underwent fibreoptic bronchoscopies and had a subsequent lung tissue biopsy taken for further T cell studies

Treatment Group	Asthmatic Participants (n=6)	
Group	Non-Atopic	Atopic
Age (Yrs)	42.8(4.9)	33.5(0.4)
Gender (M/F)	2/2	2/0
Non-Severe/Severe Asthma (n/n)	1/3	1/1
Skin prick test positive (Yes/No)	0/4	2/0
Total IgE (IU/ml)	186.7(62.7)	1922.5(1077.5)
RAST HDM (IU/ml)	-	100[(1)]

[(1)] Specific IgE tests for 1 atopic asthmatic (B04) were not available. Mean +/- Standard Error of the Mean (Std.Dev./√n)

To do so, 6 asthmatic individuals were recruited of whom 2/6 were allergic asthmatics (Table 64). Allergic asthmatics had elevated levels of total IgE, tested positive by skin prick test (>3mm^2), and had elevated levels of allergen specific IgE.

2.2.2. IL-22 expression in lung T cell lines

After isolation and culture, T cells derived from small bronchial mucosal tissue (ca 3mm^2) were assessed after polyclonal activation for their IL-22 expression potential by flow cytometry (see Methods 2.2.2, page 88).

All lung T cell lines from asthmatic and allergic asthmatics contained IL-22$^+$ T cells. The frequency of IL-22$^+$ T cells was similar in allergic and non-allergic asthmatics reaching an average of 2.4(0.9)% (data not shown).

γδ and αβ T cells

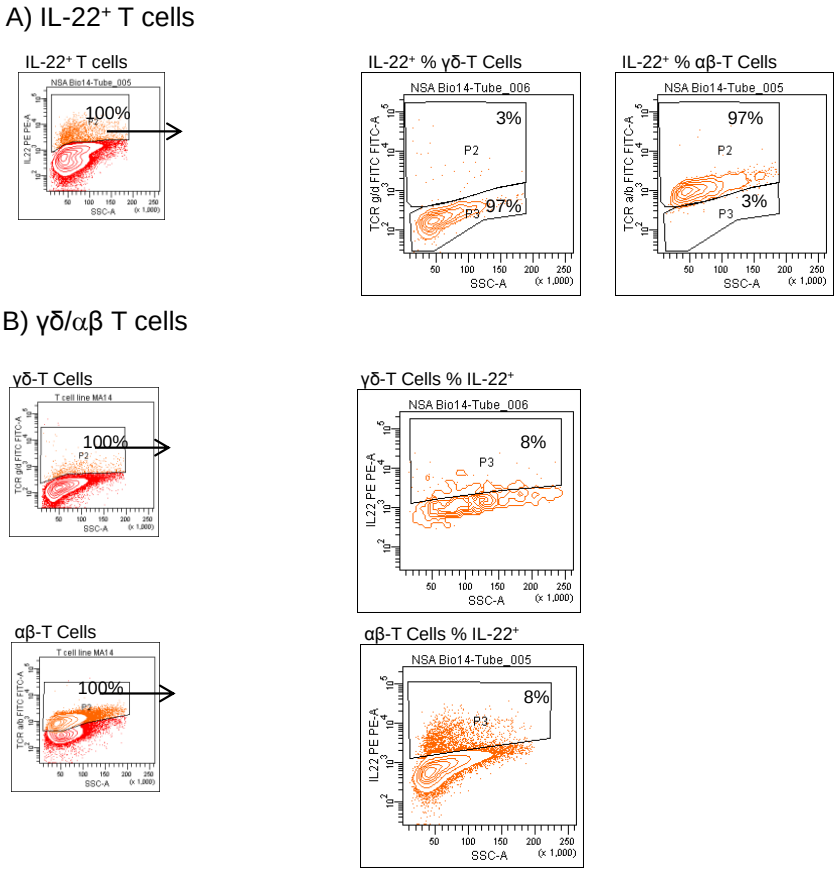


Figure 37. Flow cytometric analysis of IL-22 expression of αβ- and γδ T cell receptor positive cells from T cell lines created from bronchial tissues

Facs Plots from representative T cell line MA14

To characterise the true identity of IL-22$^+$ T cells, IL-22 was co-stained against the innate γδ- and adaptive αβ-T cell receptor.

The results showed that more than 95% of IL-22$^+$ T cells expressed the αβ T cell receptor (TCR) while less than 5% of IL-22$^+$ T cells expressed the γδ TCR (Figure 37A). Despite the huge number of αβ-TCR$^+$ IL-22$^+$ T cells, the occurrence of IL-22$^+$ T cells within each γδ- and αβ TCR$^+$ T cell population was with 8% identical (Figure 37B).

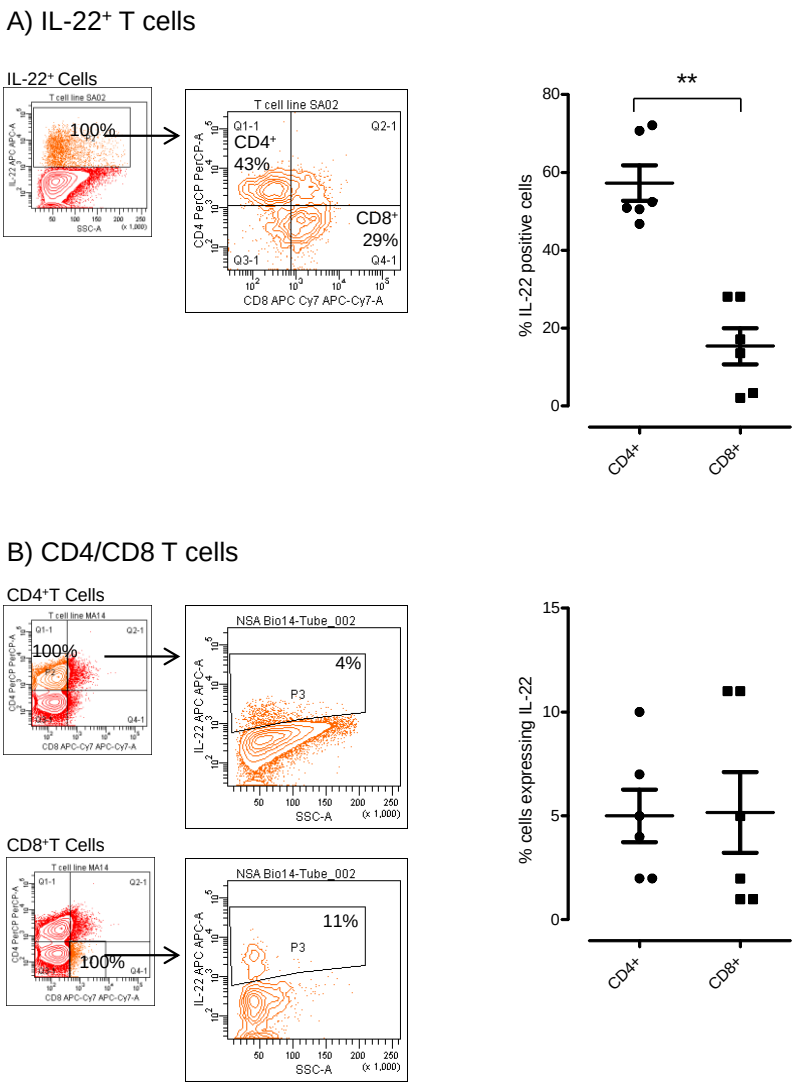


Figure 38. Flow cytometric analysis of IL-22 expression of CD4+ and CD8+ T cells from T cell lines created from bronchial tissues

A) Distribution of CD4/CD8 expression within the IL-22+ T cell population (T cell line SA02) B) Distribution of IL-22 expression within CD4/CD8 T cell populations. Statistical analysis of cumulative data (paired T-test)

Adaptive αβ T cells can be further divided into T helper cells and cytotoxic T cells. To understand which of the αβ TCR+ population accounted for the observed IL-22 expression, IL-22 was co-stained against the T helper surface marker CD4 and the cytotoxic T cell marker CD8.

The results of the flow cytometry analysis showed that IL-22+ T cells expressed significantly more CD4+ than CD8+ (p=0.005) (Figure 38A). In total, 57.3(4.5)% of IL-22+ T cells were CD4+ while only 15.4(4.6)% expressed CD8+. While CD4+ T cells accounted for the majority of IL-22+ T cells, the frequency of IL-22+ T cells within the CD4 and CD8 population was

similar (Figure 38B). IL-22 expression accounted for 5.0(1.3)% and 5.2(1.9)% of $CD4^+$ and $CD8^+$ T cells respectively.

T helper subsets

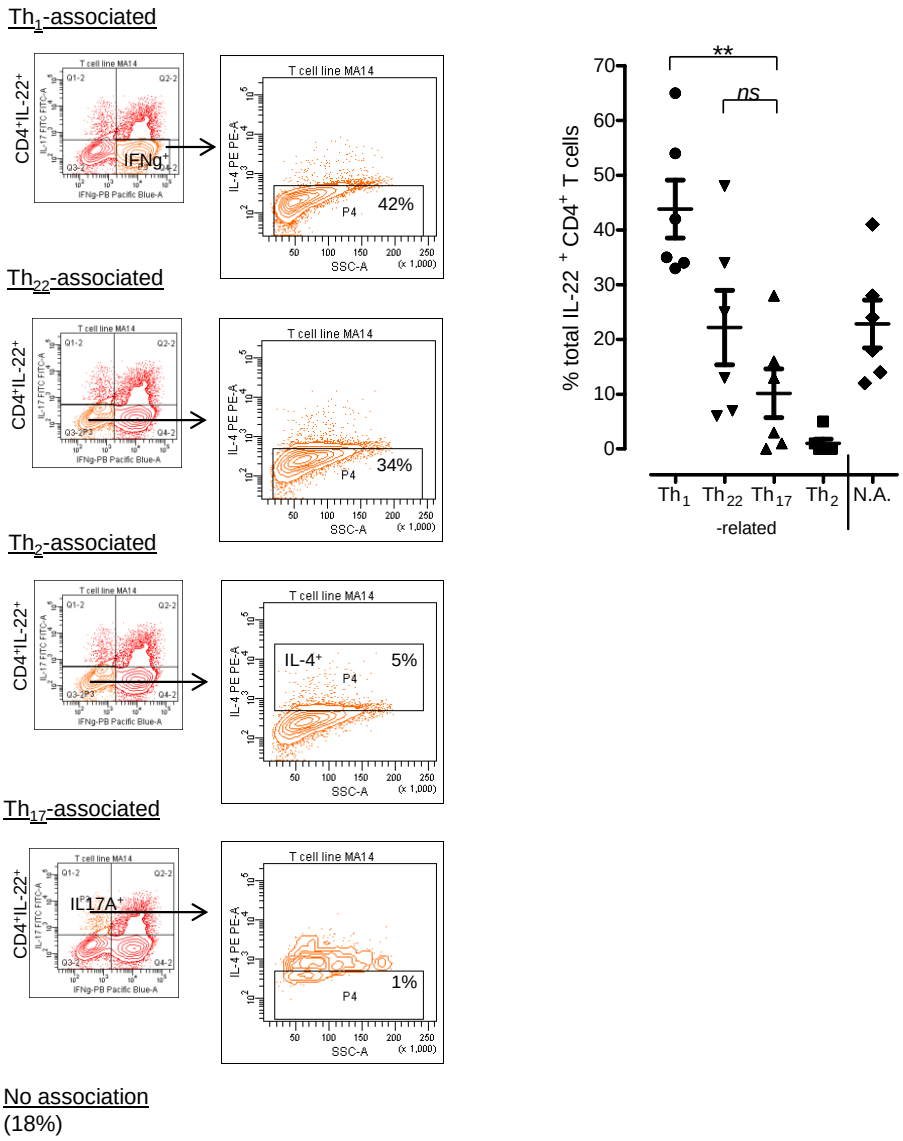


Figure 39. Flow cytometric analysis and classification of IL-22$^+$ CD4$^+$ T cells obtained from bronchial lung T cell lines into different T helper subsets

Facs plots are representative (T cell line MA14). Statistical analysis of cumulative data (Th_1 vs Th_{17} cells p=0.0088 ; paired T-test).

$CD4^+$ T cells are frequently classified into T helper subsets using their distinct cytokine expression profile. Until recently, IL-22^+ T cells were described as a derivative of Th_{17} cells. Since then IL-22 and IL-17 were found to be independently regulated (Zheng *et al* 2007) and the occurrence of significant numbers of IL-22^+IL-17^- T cells during inflammation (Scriba *et al*

2008) has led to the advocation of a new Th_{22} subset (Trifani and Spits 2010, Duhen *et al* 2009, Eyerich *et al* 2009).

To establish the true T helper subset-identify of IL-22^+ T cells within the lung T cell lines, IL-22 was stained against the lead cytokines IL-4, IFNγ, and IL-17A.

The flow cytometry analysis showed that 43.8(5.3)% of IL-22^+ T cells co-expressed IFNγ and were classified as $Th_{1/22}$ cells (Figure 39). The second largest IL-22^+ T helper population with 22.2(6.8)% were Th_{22} cells. Both $Th_{1/22}$ and Th_{22} cells were more numerous than classic $Th_{17/22}$ cells which accounted for only 10.2(4.5)% of IL-22^+ T cells. This difference was significant between $Th_{1/22}$ and classic $Th_{17/22}$ cells ($p=0.009$). The results also showed that on average 22.8(4.4)% of IL-22^+ CD4 T cells could not be associated to either of the known T helper subsets (Figure 39, 'N.A.').

2.2.3. IL-22 expression in lung T cell clones

The occurrence of large numbers of $Th_{1/22}$ and Th_{22} cells within the asthmatic T cell lines prompted us to study the different IL-22$^+$ T cells further. To investigate them, individual T cells were isolated by limiting dilution and clonally expanded *in vitro* (see Methods 2.2.3, page 89).

Flow cytometry of IL-22$^+$ T cell clones

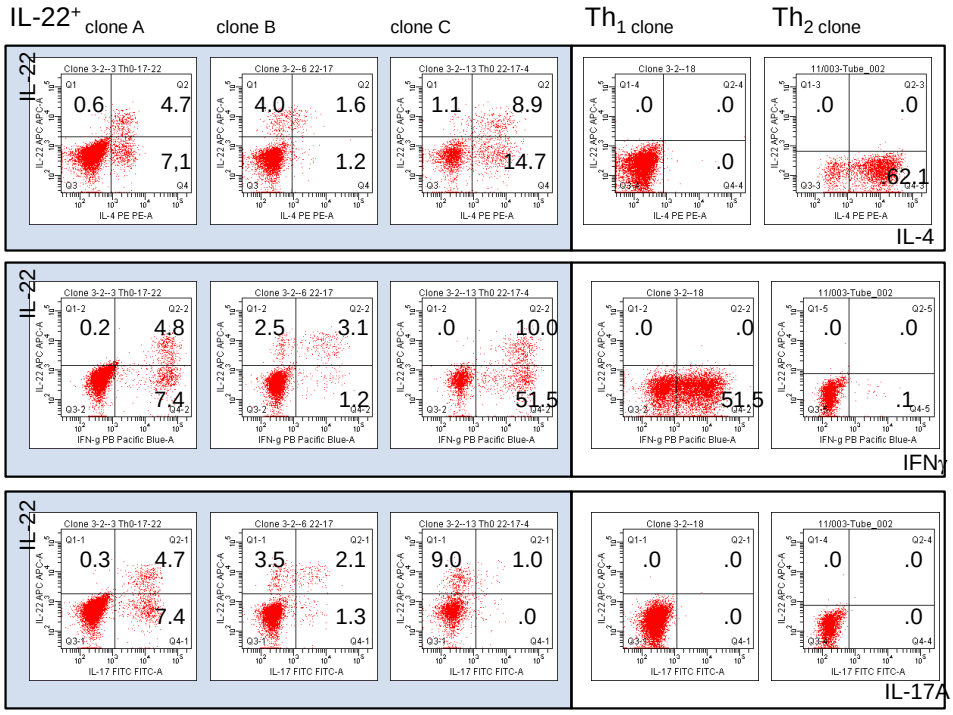


Figure 40. Flow cytometric analysis of IL-22, IL-17A, IL-4, and IFNγ co-expression in CD4$^+$ T cell clones obtained from bronchial lung mucosa

IL-22$^+$ CD4$^+$ T cell clone A (B06,ID3-2-3), B(B06,ID3-2-6), C (B06,ID3-2-13). FACS plots of Th_1 and Th_2 clones are representative

T cell clones derived from asthmatic T cell lines were assessed for a homogeneous CD4 expression and then analysed for their IL-22, IL-17A, IL-4, and IFNγ expression potential using polyclonal activation.

The results of the flow cytometry analysis showed that 3/36 T cell clones were IL-22$^+$ (Figure 40). Analysis of these IL-22$^+$ T cell clones revealed that they did not belong to the previously identified $Th_{1/22}$, Th_{22}, or $Th_{17/22}$ subset (see Results 2.2.2 Figure 39 page 136). Each of the clones was capable of expressing IL-22 together with IL-4, IFNγ and IL-17A and could thus not be clearly categorised. The remaining 33/36 T cell clones were classified as Th_1 (n=17), Th_0 (n=10), and Th_2 (n=6) T cell clones (data not shown).

2.3. Summary

Long term allergen-PBMC challenge study

- Both atopic and normal PBMC cultures responded to the allergen with increased proliferation ([methy^{3}H]-thymidine incorporation)
- After 6 days of culture IL-5 but not IFNγ was increased in atopic PBMC cultures while normal PBMC cultures showed no IL-5 or IFNγ response
- Measurement of IL-22 and IL-17 showed that IL-22 was produced by both atopic and normals after allergen challenge while IL-17 remained absent
- Flow cytometric analysis failed to detect significant levels of IL-22 in PBMC cultures at day 6. In the rare instance when IL-22 was detected, it was CD4 T cell associated and small population of co-expressing IFNγ^+ and IL-17A$^+$ but not IL-4$^+$, IL-5$^+$, or IL-9$^+$ cells could be observed

Th_{22} T cell differentiation

- Differentiation of naïve CD4 T cells derived from normal donors in Th_{22} polarising conditions (TNFα and IL-6) failed to increase the number of IL-22$^+$ T cells after culture
- The use of serum-free and serum-containing media had no influence on Th_{22} cell differentiation

Th_{22} memory T cell isolation using putative surface markers

- The putative Th_{22} markers FGFR2, TIE1 and PDGFR were all expressed by a minor population of peripheral blood CD4 T cells. While TIE1 and PDGFR were found exclusively on CD45RO$^+$ T cells FGFR2 was expressed on both CD45RO$^+$ and CD45RO$^-$ T cells
- The enrichment of FGFR2, TIE1 and PDGFR-positive CD4 T cells did not correlate with an increase IL-22$^+$ T cells
- The stimulation of peripheral blood CD4 T cells with FGF1,-2,-5 and PDGF-BB for 24 hours showed to have no influence on IL-22 cytokine expression

IL-22 expression in asthmatic lung T cell lines and clones

- Lung T cell lines from asthmatic bronchial mucosal tissue contained memory IL-22$^+$ T cells
- $\alpha\beta$TCR$^+$ and CD4$^+$ T cells were the dominant T cell population within each cell line containing the majority of IL-22$^+$ T cells. The frequency of IL-22$^+$ T cells in $\alpha\beta$ and $\gamma\delta$ as well as CD4 and CD8 T cell populations was similar
- IL-22 expression within Th_2 cells was almost always absent and the majority of IL-22$^+$ T cells belonged to the $Th_{1/22}$, and Th_{22} subset
- While Th_1 and Th_2 clones could be easily obtained, we were unable to isolate and analyse pure Th_{22} cells

3. IL-22 in human tissue regeneration and repair

Having identified IL-22 expression within the lung of allergic asthmatics after allergen challenge, we set out to identify the target cells of IL-22 and study IL-22's function on them in more detail.

3.1. IL-22 Receptor expression in human lung cell lines

The study investigated if primary lung cell lines such as bronchial epithelial cells, airway smooth muscle cells, and fibroblasts could express the IL-22 receptors IL-22RA1 and –RA2 and be used to study the function of IL-22 *in vitro*.

Protein expression by Western Blot

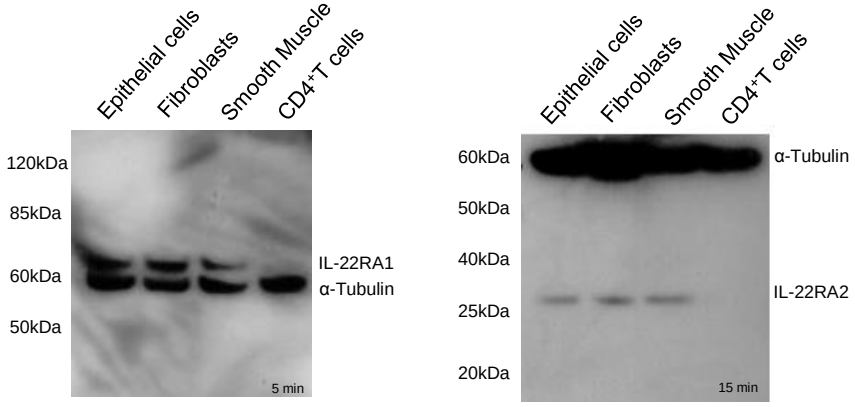


Figure 41. Western Blot analysis of IL-22 Receptor RA1 and RA2 expression in primary lung bronchial epithelial, fibroblasts, smooth muscle cell lines and peripheral blood CD4⁺ T cells

molecular weights of IL-22RA1 (63kDa), IL-22RA2 (27kDa), α –tubulin (50kDa).

To detect the constitutive expression of IL-22RA1 and –RA2 in each primary lung cell line, total cell lysates were obtained and analysed by Western Blotting (see Methods 3.1, page 92).

The Western blot results showed that IL-22RA1 was detectible in all primary lung cell lines but not peripheral blood $CD4^+$ T cells (Figure 41, IL-22RA1: visible 65kDa; calculated 63kDa). IL-22RA1 was rapidly detectable and showed similar intensity to the loading control alpha-tubulin (visible 58kDa). The intensity of the IL-22RA1 band between bronchial epithelial cells, fibroblasts, and smooth muscle cells was very similar.

IL-22RA2 was similarly detected in all three primary lung cell lines but not peripheral blood $CD4^+$ T cells (IL-22RA2:visible28kDa, calculated 27kDa). The IL-22RA2 band required 3 times longer detection times to be visible and was thus much weaker than the alpha-tubulin.

The intensity of the IL-22RA2 band between bronchial epithelial cells, fibroblasts, and smooth muscle cells was again very similar.

Surface expression by flow cytometry

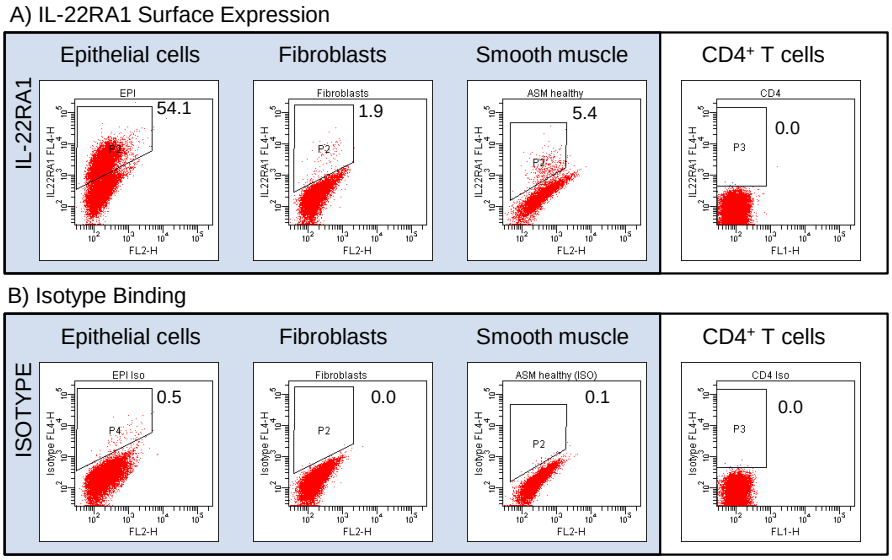


Figure 42. Flow cytometric analysis of IL-22RA1 surface expression on bronchial epithelial, fibroblast, smooth muscle lung and peripheral blood CD4⁺ T cells

A) IL-22RA1 surface stain B) Isotype control stain

To understand if the detected IL-22RA1 protein was expressed on the cell surface of bronchial epithelial cells, fibroblasts, and smooth muscle cells, each cell line was analysed by flow cytometry.

The results of the flow cytometric analysis showed that bronchial epithelial cells, fibroblasts, and smooth muscle cells tested positive for IL-22RA1 (Figure 42, $p<0.05$). The largest expression of IL-22RA1 was found in bronchial epithelial cells where 54.1(3.7)% of them were IL-22RA1 positive. In contrast, only 5.4(0.2)% of smooth muscle cells and 1.9(0.2)% of fibroblasts were capable of expressing IL-22RA1 on their surface. Peripheral blood CD4⁺ T cells, used as negative control, tested negative for IL-22RA1.

Regulation by RT-PCR

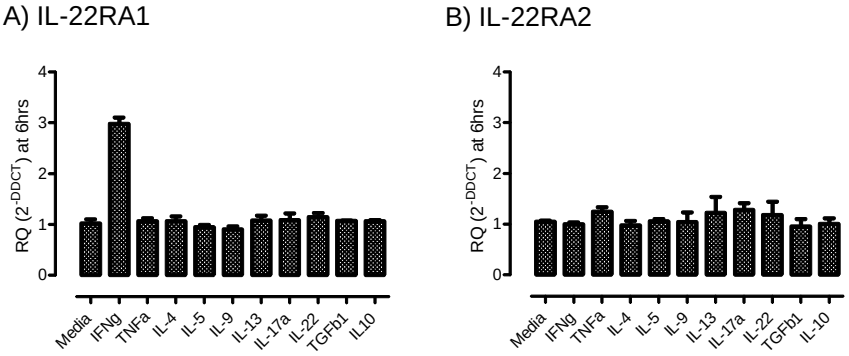


Figure 43. RT-PCR analysis of IL-22 Receptor RA1/RA2 gene expression in bronchial epithelial cells cultured in the presence of different T cell cytokines

Sample population n=3 ; Statistical test (wilcoxon)

Having identified the expression of IL-22RA1 and RA2 within primary bronchial epithelial cells, airway smooth muscle cells, and fibroblasts, the study investigated if their expression could be influenced by different T cell cytokine environments. To do so, bronchial epithelial cells were exposed to different recombinant cytokines in culture and the change IL-22RA1 and –RA2 mRNA expression analysed by RT-PCR (see methods 3.1, page 97).

The RT-PCR analysis revealed that after 6 hours of culture only IFN-γ was able to influence IL-22RA1 mRNA expression (Figure 43). IL-4, IL-5, IL-9, IL-13, IL-17A, IL-22, TGFβ1, IL-10 and TNFα had no visible effect on IL-22RA1 gene expression. In the presence of IFN-γ, bronchial epithelial cells up-regulated IL-22RA1 by 3.0(0.1)-fold. The RT-PCR analysis of IL-22RA2 showed no evidence that its gene expression was altered by the presence of IL-4, IL-5, IL-9, IL-13, IL-17A, IL-22, TGFβ1, IL-10, TNFα, or IFN-γ.

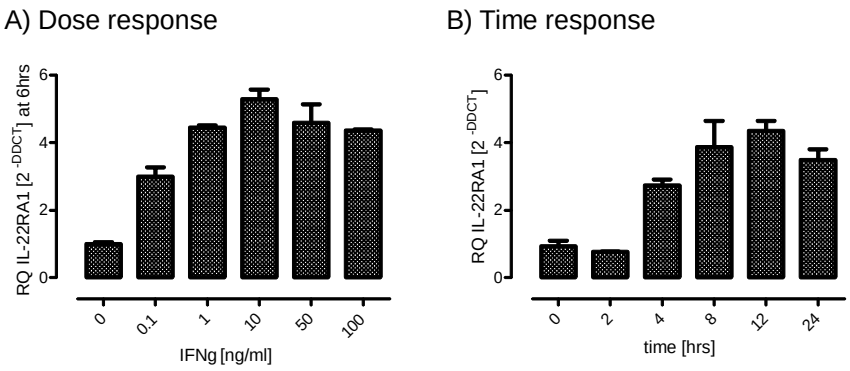


Figure 44. Dose and time response of IFNγ on bronchial epithelial IL-22RA1 expression

Sample population n=3; Statistical test (Wilcoxon)

To better understand the influence of IFN-γ on IL-22RA1 gene expression, bronchial epithelial cells were exposed to a series of IFN-γ concentrations. The results of the IFN-γ dose response showed that 0.1ng/ml was sufficient to induce IL-22RA1 up-regulation (Figure 44). The strongest up-regulation of 5.3(0.3)-fold was observed in the presence of 10ng/ml. Larger concentrations of 50 and 100ng/ml had no further beneficial effect and reduced the observed IL-22RA1 up-regulation. To understand how rapidly the IL-22RA1 up-regulation occurs and how long it could be maintained, cells were exposed to IFN-γ over a period of 24 hours and analysed. The RT-PCR results showed that IL-22RA1 up-regulation could be detected as early as 4 hours after IFNγ exposure. The IL-22RA1 up-regulation increased further reaching a maximum 4.1(0.2)-fold up-regulation at 12 hours. The IL-22RA1 up-regulation remained high and did not reduce in magnitude even after 24 hours. The analysis was widened to include airway smooth muscle cells and fibroblast. Both cell types were exposed to 10ng/ml IFNγ and their analysed by RT-PCR. The RT-PCR results showed that IFNγ was able to up-regulate IL-22RA1 mRNA expression in a similar manner not only in bronchial epithelial cells but also airway smooth muscle cells and fibroblasts (see Appendix, Figure 51, page 215).

3.2. Effect of IL-22 on bronchial epithelial cells

Epithelial cells within the lung are known to be a target of IL-22. Our primary bronchial epithelial cell line showed a high constitutive surface expression of IL-22RA1 (see Results 3.1, Figure 42, page 141) in culture and was thus an ideal model cell line to study the function of IL-22 in more detail. The main objective was to better understand the role of IL-22 in wound healing. Before any potential mechanism could be investigated, we had to confirm that IL-22 was able to improve healing in primary bronchial epithelial cells *in vitro*.

3.2.1. Wound healing

To confirm that IL-22 was able to improve healing *in vitro*, a wound healing assay commonly known as scratch assay was set up (see Methods 3.2.1, page 95). In this assay, a confluent monolayer of bronchial epithelial cells was injured or “scratched” and monitored by light microscopy over time. The difference in wound closure between IL-22 treated and untreated cell was analysed first by eye and then computer software.

IL-22 time and dose response

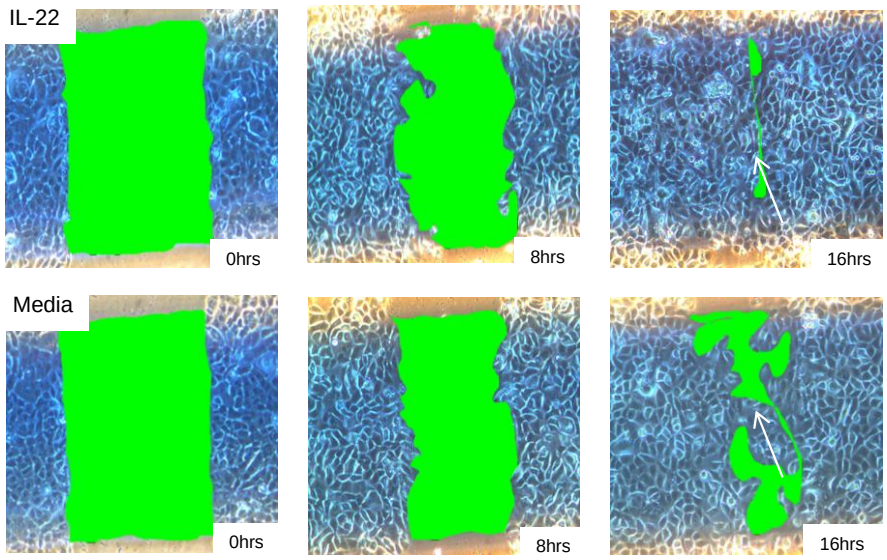


Figure 45. Light microscopic analysis of the effect of IL-22 on bronchial epithelial wound closure over time

The assessed injured surface area is represented in green. Images are representative (100x magnification).

The results of the scratch assay showed that control cells were able to almost completely close the wound 16 hours after injury (Figure 45). The difference between IL-22 treated and untreated cells was not easily detectable. While a visual difference between IL-22 and control cells was observed, the complete data set (n=9) was highly variable.

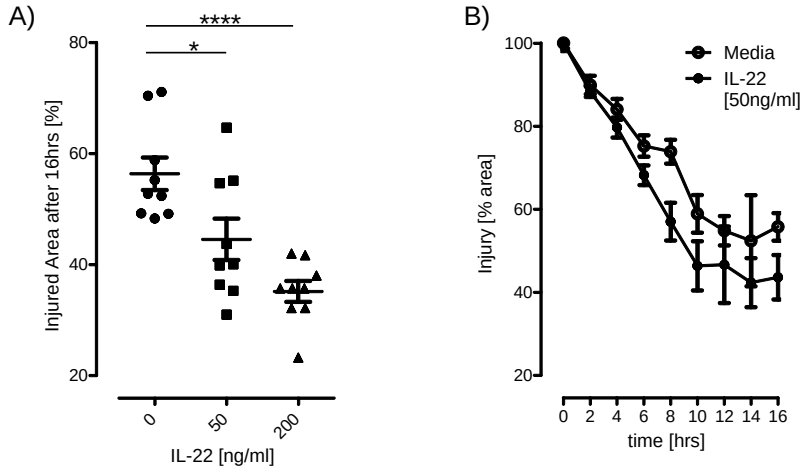


Figure 46. Software-assisted analysis of the effect of IL-22 on bronchial epithelial wound closure over time

A) 50ng/ml (p=0.0238), 200ng/ml (p=0.0001). Statistical analysis (unpaired T-test)

Therefore, the acquired data was re-analysed using specialised software. This software was able to accurately determine the surface area of the injury over time. The results showed that increasing concentrations of IL-22 of 50ng/ml and 200ng/ml significantly improved wound closure (Figure 46A). Compared to untreated cells 200ng/ml IL-22 treated cells showed a 48.8% improvement (p=0.0001). Monitoring wound closure over time (Figure 46B) showed a detectable difference between IL-22 and untreated cells as early as 4 hours after injury. Wound closure appeared to reach its peak 10 hours after injury and effectively stopped thereafter.

Effect of IL-17A on IL-22-associated wound closure

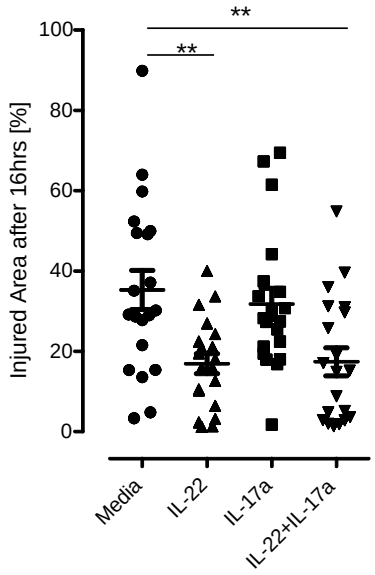


Figure 47. IL-22-associated epithelial wound closure in the presence and absence of IL-17A

IL22 (p=0.0033); IL-22+IL-17A (p=0.0090) Statistical analysis (Mann-Witney U)

The study then focused on IL-17A and if the cytokine could interfere with the IL-22-associated wound closure.

The results showed that IL-17A, on its own, had little or no effect on wound closure (Figure 47). Control cells had 35.3(4.8)% of their injury remaining while IL-17A exposed cells had 31.7(3.9)%. In addition IL-17A had little or no effect on IL-22-associated wound closure. The combination of IL-17A + IL-22 resulted in a reduction to 17.3(3.5)%. This was very similar to the 16.9(2.5)% observed in IL-22 only exposed cells.

3.2.2. Proliferation

While the function of IL-22 on epithelial wound closure could be confirmed (see Results 3.1, page 144), the underlying mechanism(s) had yet to be elucidated. Four main aspects were identified which could, in part, play a role in IL-22-associated wound closure. These were (A) Increased survival of damaged cells (anti-apoptotic) (B) Loss of cell layer integrity (loss of cell-cell contact), (C) cytoskeletal rearrangement (D) cell spreading and migration as well as (E) cell proliferation.

First, it was investigated if the presence of IL-22 could lead to increased proliferation. To assess this, IL-22 exposed bronchial epithelial cells were stained using propidium iodide acquired by flow cytometry and their individual cell cyle stages analysed (see Methods 3.1, Figure 14, page 96).

Cell Cycle Analysis

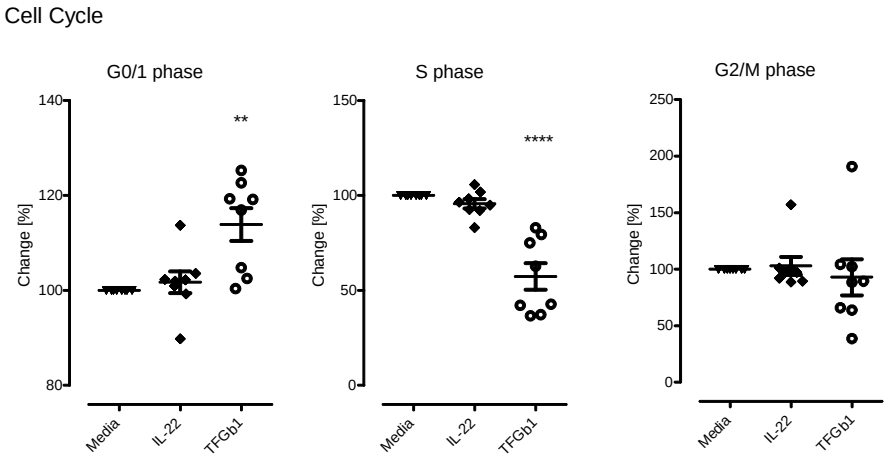


Figure 48. Analysing the effect of IL-22 on proliferation by quantifying epithelial cell cycle stages using Propidium Iodide

G0/1 phase: Media vs TGFb1 p=0.0013, S phase: Media vs TGFb1 p<0.0001 (unpaired T-test)

The analysis of the different cell cycle stages by propidium iodide is commonly used in the study of non-adherent cells such as peripheral blood mononuclear cells. After successful modifications and validation of the protocol, it was possible to stain and assess adherent bronchial epithelial cells.

The result of the flow cytometric analysis showed that IL-22 did not alter the number of resting, proliferating or dividing cells (Figure 48). The proportion of cells in G0/G1-pahse, S-phase, and G2/M-phase were almost identical between IL-22 treated and untreated cells.

TGFβ1, which is known to dampen bronchial epithelial cell proliferation, was used as a control cytokine. Compared to IL-22, TGFb1 was able to change the different cell cycle stages in bronchial epithelial cells. The cytokine reduced the number of synthesising (S-phase) ($p<0.0001$) and dividing cells (G2/M-phase) by 42.7% and 16.0% respectively while at the same time increasing the number of resting cells (G0/M) by 13.9% ($p=0.001$).

3.2.3. Gene Expression

While new functional assays were set up to investigate the contribution of IL-22 on cell survival, cell spreading and migration, IL-22 exposed cells were screened for alterations in their gene expression profile. Their profile was specifically analysed for the expression of genes associated with tissue injury and tissue repair.

Injury-associated genes

Wound healing occurs immediately at the onset of inflammation. The study, therefore, investigated if IL-22 could influence the gene expression of epithelial mediators associated with injury and inflammation. To investigate this, primary bronchial epithelial cells were cultured in presence of IL-22 and the relative change in selected gene expression analysed by RT-PCR.

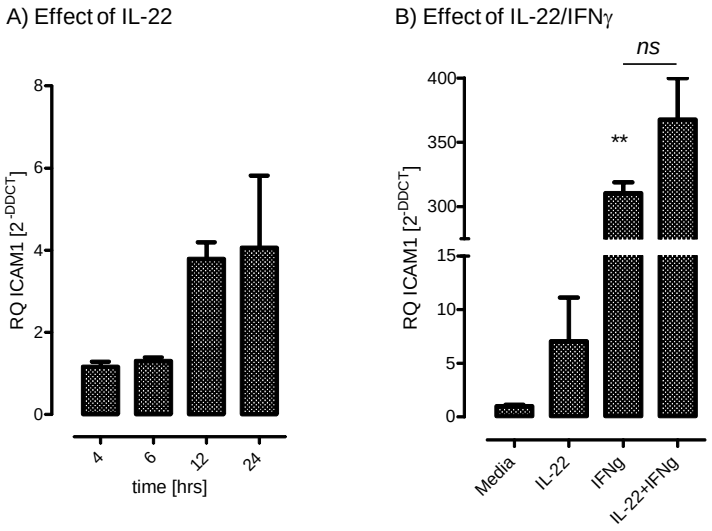


Figure 49. Regulation of ICAM-1 (CD54) gene expression by IL-22 and IFNγ in bronchial epithelial cells

B) Media vs IFNγ (P=0.0084) Sample population size n=9; Statitsical test (Wilcoxon)

The first gene to be investigated was the intracellular adhesion molecule 1 (ICAM-1). ICAM-1 through binding lymphocyte function-associated antigen 1 (LFA-1) allows the recruitment of infiltrating immune cells to the damaged and inflamed tissue. The results of the RT-PCR screen showed that after 12 hrs IL-22 was able to increase ICAM-1 expression 3.8(0.4)-fold (Figure 49). ICAM-1 expression remained high even after 24 hours with a 4.1(1.8)-fold difference.

The main inducer of ICAM-1 is the cytokine IFNγ. The study previously identified that $CD4^+$ T cells from the bronchial mucosa predominately expressed IL-22 together with IFNγ (see

Results 2.2.2 Figure 39, page 136). To test the hypothesis that IL-22 and IFNγ could act synergistically, IFNγ was included into the epithelial cell culture. The RT-PCR analysis showed that IL-22 + IFNγ strongly up-regulated ICAM-1 expression. This increase in ICAM-1 was +56.8-fold points higher than seen with IFNγ alone (310.4(8.4)-fold; p=0.0084). It was also +49.7 points higher than the sum of the individual IL-22 and IFNγ inductions together.

Table 65. Effect of IL-22 on IFNγ-regulated inflammatory gene expression in bronchial epithelial cells

Gene	IL-22	IFNγ	IL-22+IFNγ	Effect of IL-22	Synergy indicated
IL6	2.2(0.4)	17.9(2.9)	20.6(3.1)	Yes	Yes
CCL2	24.4(12.7)	1270.8(77.8)	1877.8(321.0)	Yes	Yes
CXCL9	67.3(48.6)	3796.8(456.0)	4453.2(281.1)	Yes	Yes
CXCL10	101.0(80.2)	4328.7(107.2)	6133.2(225.2)	Yes	Yes
CXCL11	78.6(64.1)	2725.9(292.4)	3553.5(333.4)	Yes	Yes

Gene expression is relative to un-stimulated cells using the ΔΔct method. Data is represented as mean (Standard error of the mean). Sample population n=3 , statistical test (wilcoxon).

Having indentified a potential synergistic interaction between IL-22 and IFNγ, 6 additional IFNγ-regulated genes were screened. These included the cytokine IL-6, and the chemokines CCL2 (MCP-1), CXCL9 (MIG), CXCL-10 (IP-10), and CXCL-11 (I-TAC). The RT-PCR analysis showed that IFNγ associated up-regulation of IL-6, CCL2, CXCL9,-10, and -11 was further enhanced in the presence of IL-22 (Table 65). The enhanced gene expression was particularly observed for CXCL-10(IP-10) and CXCL-11(I-TAC).

Regeneration and repair-associated genes

The study then went on to investigate the expression of genes associated with tissue regeneration and repair. These included matrix-metallo-proteinases, growth factors and growth factor receptors. Genes which were known to be induced by IFNγ were specifically selected. These included the matrix-metallo-proteinases MMP1, -10, the growth factors Activin (INHBA), BMP-2, ECGF-1 (TYMP), TGF-α, and VEGFc.

Table 66. Effect of IL-22 on IFNγ-regulated repair-associated gene expression in bronchial epithelial cells

Gene	IL-22	IFNγ	IL-22+IFNγ	Effect of IL-22	Synergy indicated
MMP1	2.0(0.1)	9.7(0.8)	7.7(0.6)	Yes	-
MMP10	2.0(0.3)	2.6(0.2)	2.5(0.5)	Yes	-
INHBA	1.2(0.1)	1.7(0.2)	2.5(0.3)	-	Yes
BMP2	1.1(0.0)	3.0(0.2)	3.9(0.2)	-	Yes
TYMP	1.3(0.1)	7.7(0.2)	10.5(0.9)	-	Yes
VEGFC	1.2(0.1)	2.3(0.1)	2.7(0.4)	-	Yes

Gene expression is relative to un-stimulated cells using the ΔΔct method. Data is represented as mean (Standard error of the mean). Sample population n=3 , statistical test (wilcoxon).

The RT-PCR analysis showed that MMP-1 and MMP-10 were significantly up-regulated by IL-22 (Table 66). However, the combination of IL-22 and IFNγ did not further increase MMP-1 or MMP10 expression. Overall, MMP1 was induced by 2.0(0.1)-fold by IL-22 and 9.7(0.8)-fold by IFNγ. The regulation of MMP10 was similar between IL-22 and IFNγ. IL-22 increased MMP10 by 2.0(0.3)-fold while IFNγ increased MMP10 by 2.6(0.2)-fold .

The RT-PCR analysis of the growth factors genes Activin (INHBA), BMP-2, ECGF-1 (TYMP), TGF-α, and VEGFc showed that none were altered by the presence of IL-22 alone. However, IL-22 enhanced the IFNγ-associated gene up-regulation of each gene and the further enhancement in gene up regulation reached significance for BMP2, TYMP as well as TGF-α.

As IL-22 enhanced TGF-α expression, the gene expression of its receptor EGFR and alternative ligand EGF was assessed. The RT-PCR results showed no evidence that EGFR, or EGF were regulated by IL-22, IFNγ or the combination of the two (data not shown).

3.3. Summary

IL-22 Receptor expression in human lung cell lines

- Western Blot analysis of normal bronchial epithelial, fibroblasts, smooth muscle, and T cell lysates showed that all structural cell lines but not T cells contained both IL-22RA1 and RA2 protein
- Surface analysis of IL-22RA1 expression by Flow cytometry confirmed the expression of IL-22RA1 on all structural cell lines but not T cells. Bronchial epithelial cells showed the strongest surface expression (>50%) while the expression of IL-22RA1 on smooth muscle cells and fibroblasts remained low (>5%).
- Neither IL-22RA1 nor RA2 mRNA expression was regulated by the presence of Th_2 cell cytokines. IL-22RA1 however increased in the presence of IFNγ in a dose and time dependent manner in bronchial epithelial as well as fibroblasts, smooth muscle cells

Bronchial epithelial wound healing

- The addition of IL-22 to artificially injured bronchial epithelial cells improved their wound closure ability in a dose and time dependent manner.
- This IL-22 specific effect remained unaffected by the presence of IL-17A which on its own showed to exhibit to positive or negative effect on wound healing

Bronchial epithelial proliferation

- The cell cycle analysis showed that IL-22 exposure had no influence on the number of resting, DNA synthesizing or dividing bronchial epithelial cells. TGFβ1 on the other hand significantly changed increased the number of resting cells while decreasing DNA synthesising cells

Bronchial epithelial gene expression

- Investigation of IFNγ-regulated inflammatory assoc. genes showed that IL-22 on its own was able to increase ICAM-1, IL-6, CCL2, CXCL-9,-10, and -11 expression. In addition IL-22 was able to further enhance the strong induction of each gene by IFNγ.
- IFNγ-regulated repair assoc. genes showed only in part to be induced by IL-22 alone. MMP1 and MMP10 were both induced by IL-22 but showed no further synergy in the presence of IFNγ. IFNγ-associated growth factors were not regulated by IL-22 alone. However in combination with IFNγ, IL-22 was able to enhance the observed growth factor up regulation.

Discussion

The investigation of “IL-22 in allergy and asthma” focused on the expression of the cytokine during human allergic inflammation, its association with T cells, and its effects on structural cells in relation to tissue regeneration and repair.

1. IL-22 expression in allergic inflammation

The hypothesis that “IL-22 is a T cell cytokine that is expressed during human allergic inflammation” was tested in a clinical study, exposing allergic asthmatics and normal healthy individuals to inhaled allergen using a segmental bronchial lung challenge protocol.

1.1. Local bronchial segmental allergen challenge

Segmental bronchial allergen challenge resulted in an early and late phase asthmatic response, including late falls in FEV_1 in all asthmatics but not normal control subjects. Analysis of the bronchoalveolar lavage showed late increases in eosinophils, the Th_2 cytokine IL-5, but not the Th_1 cytokine IFNγ. Within the context of a human Th_2 response in the lung, the relevance of IL-22 and its decoy receptor IL-22RA2 were explored. Both IL-22 and IL-22RA2 were significantly increased while the related cytokines IL-17A/F remained low and unchanged. Thus, in allergic asthmatics, IL-22 and IL-22RA2 were released alongside IL-5 independent of IFNγ and IL-17A/F during the course of the human allergic reaction.

In comparison to natural allergen exposure, the immune response elicited during experimental bronchial allergen challenge was shown to be very similar (de Bruin-Weller *et al* 1999). The added benefit of selecting an experimental challenge model was that it could be carried out under safe, controlled and standardized conditions (Gauvreau *et al* 2007, Julius *et al* 2008, Kämpe *et al* 2010) reducing the variability of the allergic immune response (Arvidsson *et al* 2007). As the primary allergen, Timothy grass (*Phleum pratense*) was selected as it is relevant in both allergy (e.g. allergic rhinitis) and asthma (Corren *et al* 1997). While alternative allergens such as house dust mite are more reflective of chronic perennial asthma, *Phleum pratense* allowed us to study the allergic response out of season (in the absence of any natural exposure) with a clean baseline. An important limitation was that, owing to difficulty in recruitment, we had to rely on a small study group consisting of 16 allergic asthmatics and 3 normal healthy individuals.

The study of the cellular and soluble composition of bronchoalveolar lavage is dependent on the diffusion of cells and mediators into the bronchial lining fluid. In general, bronchoalveolar lavage was found to be reflective of the underlying immune response within the tissue (Connett *et al* 2000) and is, therefore, commonly used in the study of airway inflammation

(Meyer *et al* 2007). The large volumes of saline (100ml) used to obtain broncho alveolar lavage can often be a limitation in the analysis of cytokines as it may dilute the concentration of the mediators below the detection limit of conventional assays. Having been able to detect IL-22 alongside IL-5 in the un-concentrated bronchoalveolar lavage underscored the potential importance of this cytokine during the allergic immune response. As the sampling of bronchoalveolar lavage is an invasive procedure, only two essential time points, namely before and 24 hours after challenge, were investigated. Any important differences in IL-5, IL-22 and IL-22RA2 expression dynamics associated with either the early, late or resolution phase of inflammation could, therefore, not be delineated.

IL-22RA2 could only be measured within the bronchoalveolar lavage after developing a novel ELISA. A sandwich ELISA design was selected to ensure high sensitivity of this assay. This required the use of two antibodies raised against alternative target epitopes of IL-22RA2. The selection was restricted as only one antibody, raised against an alternative IL-22RA2 peptide, was commercially available. The validation of this alternative antibody (T16) by direct ELISA (see Methods 1.1., Figure 7-9, page 72) showed that it had to be used in much higher concentrations than the common IL-22RA2 (N16) antibody. Given this, it was selected to be the detection rather than the capture antibody within the ELISA design. The ELISA signal could not be further amplified using a secondary anti-immunoglobulin antibody as both capture and detection antibodies were raised in the same animal species. Instead, the detection antibody was labelled directly with biotin (see Methods 1.1 page 72) to avoid non-specific detection.

The segmental bronchial allergen challenge data reflected the common observation that allergen exposure in allergic asthmatics caused a reduction in lung function, increased eosinophil infiltration, and Th_2 but not Th_1 cytokine expression (Teran *et al* 1999, Johansson *et al* 2008, Bentley *et al* 1993, Thunberg *et al* 2010, van de Pol *et al* 2012, Lilly *et al* 2001, Pilette *et al* 2004). Within this Th_2-dominated environment, the detection of IL-22, in the absence of both IL-17 and IFNγ, was significant. The absence of IL-17 and IFNγ after bronchial segmental allergen challenge has recently been confirmed by two independent studies (Barlow *et al* 2011, Newcomb *et al* 2012). Yet, like many studies of Th_{17} cells, these studies failed to address the expression of IL-22. The observation was reminiscent of chronic atopic dermatitis where IL-22 was released in the absence of IL-17 and IFNγ (Eyerich *et al* 2009). What is known is that, in asthma, the allergic T helper cell response can shift and become more heterogeneous over time showing signs of both Th_1 and Th_{17} responses (Cembrzynska-Nowak *et al* 1993, Randolph *et al* 1999, Huang *et al* 2001, Chakir *et al* 2003, Cho Stanciu *et al* 2005, Morgan *et al* 2005, Koch *et al* 2006, Molet *et al* 2001, Wong *et al*

2001, Barczyk *et al* 2003, Hashimoto *et al* 2005). These changes in the helper cell response away from the Th_2 dominated environment remain controversial and are hypothesised to be driven by factors other than the allergic component of asthma (Lajoie *et al* 2010, Maxmen *et al* 2011).

The documented increase of IL-22RA2 was novel. Published data on human IL-22RA2 protein expression are still not available until to date. It has been known that the recombinant IL-22RA2 protein was able to bind IL-22 and block its signalling (Xu *et al* 2001, Dumoutier *et al* 2001, de Moura *et al* 2009, Jones *et al* 2008, Wolk *et al* 2007, Wu *et al* 2008). The only two murine studies documenting a change in IL-22RA2 (mRNA) expression showed a decrease during acute inflammation and an increase days later (Wolk *et al* 2007, Wilson *et al* 2010) indicating a potential role of IL-22RA2 during resolution of inflammation.

On the basis of these findings, it was hypothesised that the strong increase in IL-22 was related to a functional role during allergic inflammation in the lung. The occurrence of IL-22 outside the classic Th_1/Th_{17} environment supported recent findings that a novel Th_{22} subset may participate during allergic inflammation. The presence of IL-22RA2 in bronchoalevolar lavage 24 hours after challenge indicated that the function of IL-22 had been actively suppressed at one point in time. It is possible that IL-22RA2 may have been produced during the resolution of inflammation after the early and late phase response had peaked.

In conclusion, the expression of IL-22 during late asthmatic responses, in the context of a Th_2-dominated environment, supported a potential role for IL-22 in human allergic asthma and raised the question of the cell source of IL-22, expression dynamics of IL-22 and the function of this cytokine during allergic inflammation. To identify individual cells expressing IL-22, IL-22RA2 and IL-22RA1, in the context of late allergic inflammation, human skin biopsies taken after intradermal allergen challenge were obtained and analysed by immunohistochemistry.

1.2. Intradermal allergen challenge

Intradermal allergen challenge caused an early and late phase skin reaction in all atopic allergic but not in normal controls. The obtained allergen challenged tissue showed an increase in both $CD3^+$ T cells and eosinophils 8 hours after challenge. Within the context of a human allergic immune reaction n the skin, the expression of IL-22, IL-22RA1, and IL22RA2 was explored. The tissue was stained using 3 different anti-human IL-22 antibodies. These antibodies had been validated using paraformadehyde-fixed cytospin preparations of activated peripheral blood memory T cells but failed to produce a visible stain in similarly fixed skin tissue. One of the antibodies, however, was able to stain $IL\text{-}22^+$ cells in acetone-

fixed skin. The number of IL-22$^+$ cells was high both in diluent and allergen challenged skin and showed no co-staining to either CD3 or IL-22RA1. The staining and distribution of IL-22$^+$ cells showed strong similarities to individual and clustered CD31$^+$ cells. The study of the IL-22 receptors IL-22RA1 and RA2 showed a wide expression throughout the tissue which remained unchanged after allergen challenge. The expression did not correlate to resident or infiltrating immune cells including T cells, B cells, macrophages, mast cells, neutrophils, and eosinophils. The majority of IL-22RA1$^+$ and RA2$^+$ cells were located within the connective tissue co-stained to vimentin. Within the connective tissue, early (HSP47$^+$) but not late (αSMA$^+$) fibroblasts co-expressed IL-22RA2. Thus, despite the successful induction of a late allergic immune response in the skin, the identity of IL-22$^+$ cells could not be convincingly investigated. The expression of IL-22RA1 and -RA2 remained unchanged after allergen challenge and both showed a wide expression throughout the tissue, on cells of mainly mesenchymal origin.

The intradermal allergen challenge was selected as it was safer to perform (than a segmental allergen challenge) and allowed the recruitment of volunteers consenting to have a local punch biopsy taken. The intradermal challenge of an inhaled allergen like *Phleum pratense* can sometimes elicit a weaker (Wood *et al* 1999, Tamura *et al* 1991) but otherwise similar allergic immune response to inhalation (Frew and Kay 1988). An additional benefit was that the immune response within the skin is largely unperturbed by other environmental influences (Durham *et al* 1991, Charlesworth *et al* 1991, Gaga *et al* 1991, Bierman *et al* 1990).

To detect individual IL-22, IL-22RA1 and IL-22RA2 cell populations within the skin, immunohistochemistry was used. As the tissue represents a complex mixture of antigens, frequently altered by storage and fixation, each antibody had to be thoroughly validated before use. As more than half of the antibodies were polyclonal, even weakly isotype-control-stained areas as well stained cells without clear cell morphology or clear DAPI-stained nuclear centre, were categorically excluded. As soluble mediators such as IL-22 are not as easily detected (compared to surface receptors), paraformaldehyde-fixed tissue sections were initially utilised. Paraformaldehyde is believed to better retain soluble mediators within cells (Naish *et al* 1989) and the validation showed that all 3 anti-human IL-22 antibodies were able to be detected in a small number of IL-22$^+$ cells in paraformaldehyde-fixed polyclonally activated peripheral blood memory CD4$^+$ T cells (see Methods 1.2, Figure 10, page 78). The fact that , IL-22 was not detected in paraformaldehyde but unexpectedly in acetone-fixed tissue, was therefore usual. Compared to paraformaldehyde, acetone may break down cell membranes leading to an increased loss

of soluble antigens from the tissue. Despite the positive staining of individual IL-22^{+} cells, the overall staining for IL-22 in acetone-fixed tissue appeared non-specific and largely confined to blood vessels. One explanation may be that acetone pre-treatment may have modified an endothelial antigen to become cross-reactive with IL-22 protein to become non-specifically recognised by the IL-22 antibody. The absence of discrete IL-22 stained cells in paraformaldehyde-fixed tissue does not necessarily confirm the absence of the cytokine within the tissue. T cells have very little cytoplasm and while they may produce cytokines in large amounts, the cytokine is synthesised and released rather than stored within intra-cytoplasmic granules, such that cytokine may be undetectable within cells. Previous studies have circumvented this problem by use of *in situ* hybridisation which detected intracellular cytokine mRNA in T cells (Bentley *et al* 1993, Robinson *et al* 1996, Ying *et al* 1997). However, the method of *in situ* hybridisation employed in these studies was complex and time-consuming and required the use of S^{35}-labelled probes derived from cytokine cDNAs as templates, in order to achieve the necessary sensitivity and specificity to detect intracellular cytokine mRNAs. The method has largely been discontinued due to poor availability of S^{35} and the relative insensitivity of enzyme-linked detection methods for detecting cytokine mRNA in tissues.

The investigation of IL-22RA1 and IL-22RA2, however, provided new insights concerning their expression in tissues and during allergic inflammation. The study initially focused on immune cells as newer studies had identified an up regulation of IL-22RA1 in different environmental contexts (Dhiman *et al* 2009, Schnyder *et al* 2010). The investigation, however, showed that immune cells did not express IL-22RA1 either before or during experimentally induced cutaneous late allergic inflammation. The results were, thus, in keeping with previous studies that showed IL-22RA1 to be generally absent in immune cells (Dumoutier *et al* 2000, Kotenko *et al* 2001, Wolk *et al* 2004, Whittington *et al* 2004, Kunz *et al* 2006, Wolk *et al* 2008). The primary focus remained on IL-22RA2 in view that IL-22RA2 mRNA had been identified predominantly in immune cells in previous reports (Xu *et al* 2001, Dumoutier *et al* 2001, Wei *et al* 2003, Wolk *et al* 2004, Whittington *et al* 2004). However, no evidence could be found that either resident or infiltrating immune cells expressed IL-22RA2.

Both IL-22RA1^{+} and IL-22RA2^{+} cells showed a stronger association with structural cells rather than with immune cells. Within the skin, the only structural cell known to express IL-22RA1 were keratinocytes (Wolk *et al* 2004, Kunz *et al* 2006, Sa *et al* 2007, Boniface *et al* 2007, Ma *et al* 2008, Pene *et al* 2008, Kragstrup *et al* 2008, Nograles *et al* 2008, Wolk *et al* 2009, Eyerich *et al* 2009). The wide expression of both IL-22RA1 and IL-22RA2 throughout the dermis was a novel finding.

The majority of IL-22RA1$^+$ and IL-22RA2$^+$ cells were vimentin$^+$ and belonged to cells of mesenchymal origin. Synovial fibroblasts were previously shown to express both IL-22RA1$^+$ and Vimentin$^+$ in culture (Ikeuchi *et al* 2005) and ganglia and myofibroblasts were capable of responding to IL-22 *in vitro* (Andoh *et al* 2005, Wolk *at al* 2009, Hosokawa *et al* 2009). While we could not use dual staining methods to investigate IL-22RA1, due to conflicting antibody pairing, we were able to investigate the expression of IL-22RA2 which showed an association to early HSP47$^+$ but not late αSMA$^+$ fibroblasts.

On the basis of these findings, it remains unanswered whether IL-22 was released by T cells and if these cells were associated either with the Th_2 or distinct Th_{22} T helper cell subset. While the identity of IL-22$^+$ cells remained elusive, our data indicated that a wider range of IL-22 target cells existed than was previously thought. Both IL-22RA1$^+$ and IL-22RA2$^+$ cells occurred mainly in the connective tissue, which contains several stem cell-like progenitor cells as well as fibroblasts, strengthening the hypothesis that IL-22 was related to tissue regeneration and repair. Both multi-potent precursor cells as well as fibroblasts have not been well researched in connection with IL-22. The limited data on fibroblasts expressing IL-22RA1 (Ikeuchi *et al* 2005, Andoh *et al* 2005, Wolk *et al* 2009, Hosokawa *et al* 2009) and our observation of IL-22RA2$^+$ expression by HSP47$^+$ fibroblasts suggests that early fibroblasts represent an *in vivo* target for IL-22, possibly involved in repair and/or remodelling processes.

Since it was not possible to link IL-22 expression to T cells in tissues, possibly related to the limitations of the available methodology and the fact that cytokines are secreted rather than stored by T cells, cytokine induction during allergen-stimulated T cell responses *in vitro* was studied. In addition, pulmonary fibroblasts were included to be studied later on as a target of IL-22.

2. IL-22 expression in T cells

To test the main hypothesis that “Interleukin-22 is a T cell cytokine produced during allergic inflammation” as well as the hypothesis that “Interleukin-22 is produced by distinct Th_{22} cells as well as other T cell subsets (Th_1,Th_2,Th_{17})”, several *in vitro* studies were set-up.

2.1. Peripheral blood mononuclear cell cultures

Using peripheral blood mononuclear cells, the occurrence of IL-22 as well as expression profile of IL-22$^+$ T cells after allergen stimulation was analysed. In parallel, the study aimed to investigate IL-22$^+$ T cells in more detail by obtaining them through de-novo Th_{22} differentiation as well as memory Th_{22} cell isolation using novel surface receptors.

2.1.1. Long term allergen-stimulated peripheral blood mononuclear cells

Allergen stimulation of PBMCs from both atopic and non-atopic subjects resulted in increased proliferation. This proliferative response was numerically but not significantly increased in PBMCs derived from allergic subjects. Culture supernatants obtained after allergen stimulation contained increased levels of IL-5 selectively in allergic individuals whereas modest increases in IL-22 were observed in cultured PBMCs from both atopic and normal subjects. IFNγ and IL-17A/F levels remained low before and after allergen challenge in both atopic and normal subjects. Flow cytometric analysis of IL-22^+ PBMCs revealed little or no IL-22 expression at day 6 post allergen stimulation. In the rare instance where IL-22 was detected, it co-localised to $CD4^+$ cells within the lymphocyte population. These $CD4^+$IL-22^+ cells did not co-express Th_2-cytokines IL-4, IL-5, or IL-9 whereas a small percentage co-expressed IL-17A and IFNγ.

PBMCs are commonly used to facilitate the antigen presentation and T cell activation *in vitro* (Till *et al* 1998). In this study, 2 out of 3 of PBMC cultures from allergic subjects but not normal controls responded to allergen with production of IL-5 which was significant. In contrast, a modest induction of IL-22 was not only observed in PBMC cultures from atopics but also from normal subjects. These findings *in vitro* are in contrast to those, following allergen provocation in the bronchi, where no IL-22 was detected in BAL in normal controls after allergen challenge. This could possibly be explained by an intact epithelial barrier which may, *in vivo,* greatly reduce allergen exposure, uptake and T cell priming in non-atopic normal subjects compared to allergic asthmatics (Hogg and Eggleton 1984, Laitinen *et al* 1985, Montefort *et al* 1992).

To identify the source of IL-22, the cultured cells were analysed by flow cytometry. An important limitation was that intracellular cytokines could only be detected after strong TCR activation and blockade of protein release. The strong TCR activation, achieved by using PMA and ionomycin, inevitably caused the activation of all T cells within the culture and not just allergen-specific T cells. To limit the contribution of non-specific T cells within each culture, the latest available time point (day 6) was selected to allow optimal polarisation and expansion of allergen-specific T cells in culture. However, it was apparent after the data was analysed that the IL-22 production could have peaked before day 6. Analysis by ELISpot could have possibly resolved this although ELISpot assays for IL-22 were not commercially available at the time.

The *in vitro* challenge data reflected previous studies in which *Phleum pratense* elicited a Th_2-type response characterised by the selective release of IL-5 and IL-13 in the absence of IFNγ and IL-17 (Till *et al* 1997).The increase of IL-22 independent of Th_1 and Th_{17} cytokines,

in the context of an allergen challenge, confirmed the previous *in vivo* data. The expression of IL-22 in PBMCs from both allergic and normal control subjects suggested that the expression of IL-22 was not linked to atopy *per se*. This finding is supported by two large scale microarray studies of allergen stimulated atopic and normal PBMCs (Liu *et al* 2005 GSE1964, Bosco *et al* 2009 GSE14908). In both datasets, there was no differential expression of IL-22 detected in PBMCs from atopic individuals compared to those obtained from normal subjects.

The *in vitro* allergen challenge provided important clues regarding the role of IL-22 during allergic inflammation. While the study supported the expression of IL-22 during allergic inflammation, it provided evidence that it may be neither atopy nor Th_2-related.

The induction of a cytokine in response to allergen in both atopic and normal PBMCs cultures draws parallels to IL-10. It, too, was released independent of the atopic status (Abbal *et al* 1998, Böttcher *et al* 2003, Bullens *et al* 2005) and has also been documented to be increased after challenge *in vivo* (Robinson *et al* 1996, Matsumoto *et al* 2004). Both, IL-10 and IL-22 are structurally related (Dumoutier *et al* 2000, Kotenko *et al* 2001) and their induction is both dependent on aryl hydrocarbon receptor (AHR) and Notch signalling (Veldhoen *et al* 2008, Quintana *et al* 2008, Veldhoen *et al* 2009, Gandhi *et al* 2010, Apetoh *et al* 2010, Mukherjee *et al* 2009, Alam *et al* 2010). Both cytokines, however, target different cells (IL-10 immune cells, IL-22 structural cells) and, thus, have little functional overlap (Lecart *et al* 2002, Wolk *et al* 2005). Yet, in mice, IL-22 was shown to suppress antigen-induced immune responses and eosinophilic airway inflammation via an IL-10-associated mechanism (Nakagome *et al* 2011). If IL-22 has a similar regulative and protective function, it would be, opposite to IL-10, related specifically to structural cells. Combined with the hypothesis that IL-22 is involved in tissue injury and repair, it would suggest that IL-22 could be released to prevent and or limit tissue damage during acute inflammation.

In conclusion, the *in vitro* allergen challenge model confirmed the expression of IL-22 after allergen stimulation but indicated that IL-22 may have a more general role not linked to atopy or allergic inflammation *per se*. The FACS analysis did reveal that a small percentage of IL-22 was detectable and co-localised to $CD4^+$ T cells. Of these, a small percentage could co-express Th_1 (IFN-γ) and Th_{17} cytokines (IL-17A), possibly indicating a closer developmental relationship of IL-22-producing cells to these T cell lineages rather than Th_2 cells. To better understand the relationship of different T helper subsets and the transcriptional regulation of Th_{22} cells, IL-22 producing T cells needed to be obtained, isolated and studied in more detail. To do so, a newly described Th_{22} differentiation protocol (Duhen *et al* 2009) as well as novel Th_{22} surface receptors and their ligands (Eyerich *et al* 2009) were utilised.

2.1.2. Polarised naive T cells

In this study, the differentiation of naive T cells using the newly described Th_{22} protocol (Duhen *et al* 2009) in my hands showed no evidence of an increase in IL-22^+ T cells. Neither the combination of the polarising cytokines IL-6 and TNFα nor their individual use increased IL-22 expression in culture. This was true whether the polarisation was carried out using serum-free or serum-containing media. It is noteworthy to mention that the short period of 7 day differentiation and 7 day expansion (in IL-2 containing media) was unusual as *in vitro* T helper cell differentiation is generally regarded as inefficient, requiring several rounds of re-stimulation and may take up to 4 weeks of culture (Cousins *at al* 2002).

TNFα is not commonly used in T cell differentiation and showed no effect on IL-22 expression (Kreymborg *et al* 2007, Volpe *et al* 2009). The only functional evidence was observed when TNFα decreased IL-17A expression in Th_{17} cultures (Baba *et al* 2010) and blocked T regulatory cell development (Ma *et al* 2010). TNFα may, thus, be linked to IL-22 and Th_{22} cells by blocking other developmental pathways. This, however, remains to be established. Similarly the link between IL-6 and IL-22 is controversial. Some studies reported a mild induction of IL-22 in IL-6 containing Th_{17} polarising conditions (Chung *et al* 2006, Zheng *et al* 2007). The use of IL-6 within T cell differentiation cultures was shown to be essential for IL-17 but not IL-22 (Kreymborg *et al* 2007, Volpe *et al* 2009). Similarly, IL-6 knockout mice expressed normal levels of IL-22 while IL-17 was greatly reduced (Zheng *et al* 2007, Zenewicz *et al* 2008, Siegemund *et al* 2009). IL-6, like TNFα, could only be involved indirectly in the induction of IL-22. One mechanism hypothesised, but not proven, was the up-regulation of the IL-23 receptor by IL-6 (Nurieva *et al* 2007). IL-23, produced mainly by dendritic cells (Siegemund *et al* 2009), was shown to be essential for the induction of IL-22 (Zheng *et al* 2007, Chung *et al* 2006, Kreymborg *et al* 2007, Volpe *et al* 2009). The use of IL-23 blocking antibodies (Liang *et al* 2007) and IL-23 knockout mouse (Aujla *et al* 2008, Siegemund *et al* 2009, de Luca *et al* 2010) confirmed a significant reduction of IL-22 (but not IL-17), when IL-23 is absent. The IL-23 receptor is not expressed initially on naive T cells (Wilson *et al* 2007) but its expression is triggered by co-stimulatory molecules during antigen presentation (de Wit *et al* 2011). Despite the strong link, IL-23 was not included within in the Th_{22} protocol.

An important aspect which was not disclosed in the original Th_{22} protocol (Duhen *et al* 2009) was the type of media used. The media can be important in the differentiation of T cells and, in particular, IL-22^+ T cells. One example is the use of Iscove's modified Dulbecco medium (IMDM) which enhanced IL-22 expression during Th_{17} cell differentiation (Veldhoen *et al* 2008). This media was shown to be rich in aromatic amino acids and natural aryl

hydrocarbon receptor (AHR) ligands. AHR ligands and AHR signalling was found to be essential for IL-22 but not IL-17 expression (Veldhoen *et al* 2008 & 2009, Trifani and Spits 2010).

The study results, as well as previous historic evidence, raised questions concerning the feasibility of *in vitro* differentiation of Th_{22} cells with TNFα and IL-6 alone. While it was not possible to exclude a putative role for TNFα and IL-6, it was evident that other factors must be involved. Despite the availability of additional strategies, (e.g. inclusion of AHR ligands, and/or IL-23) it was uncertain that the additional time spent would result in a working Th_{22} differentiation protocol. The other option to study IL-22 expressing T cells and their relation to other T helper subsets is to obtain already differentiated memory $CD4^+$ T cells from T cells obtained from peripheral blood or from bronchial tissue from asthmatic subjects.

2.1.3. Memory T cells

The use of novel surface receptors FGFR2, TIE1 and PDGFR to enrich Th_{22} cells from the peripheral blood was evaluated. These receptors, which had been identified on Th_{22} clones within the skin, were expressed by a minor population of peripheral blood $CD4^+$ T cells. The receptors, TIE1 and PDGFR, were found exclusively on $CD45RO^+$ T cells. FGFR2 expression was, however, found on both $CD45RO^+$ and $CD45RO^-$ T cells. The enrichment of FGFR2, TIE1 and PDGFR-positive T cells showed no selective increase in IL-22^+ T cells. The enrichment of IL-22^+ T cells, in general, was possible. Using CCR4, -6, and -10, the number of IL-22^+ T cells increased mildly in CCR10 and significantly in CCR6 -enriched T cell populations. In addition, the activation and co-culture of $CD4^+$ T cells in FGF- and PDGF-receptor ligand containing media (FGF-1,-2,-5 and PDGF-BB) neither influenced IL-22, nor IL-17A, IL-4, IFNγ, IL-10 or TGFβ1 mRNA expression. Thus FGFR2, PDGFR and TIE1 expressing T cells showed no potential to be used to selectively enrich Th_{22} cells from the peripheral blood.

To isolate receptor positive T cells, flow assisted cell sorting was initially employed. While promising results were obtained (see appendix, Figure 50, page 214), this method had to be abandoned due to frequent and multiple technical problems with the FACS sorting machine. For this reason, enrichment was carried out using magnetic bead separation. The magnetic bead isolation resulted in 80-90% purity of receptor-positive cells slightly less effective than the greater than 95% achieved using flow assisted cell sorting. Another limitation of magnetic separation was that only one receptor could be isolated at any one time. Despite this, it was highly reliable and reduced cell stress and cell damage, an advantage over FACS-sorted cells, which, on polyclonal activation, showed a high mortality.

Prior to their identification on skin-derived Th_{22} cells, FGFR2, PDFR and TIE had never been linked to IL-22 expression before. In addition, their expression on T cells themselves is not well documented. FGFs, which are potent growth factors released during injury and repair had been shown to enhance TCR signalling and NFAT activation of $FGFR1^+$ $CD4^+$ T cells (Byrd *et al* 2003). The expression of FGFR2 not only on memory but also naive T cells may, thus, indicate that the receptor has a similar role to FGFR1 and may facilitate T cell activation by providing a context-dependent danger signal. Yet, its expression on naive T cells also indicated that the receptor is less likely to be a specific marker of Th_{22} cells. PDGFR is another important growth factor receptor expressed during injury and repair but, so far, has only been identified on structural cells (Tan *et al* 2006, Gabrielli *et al* 2007). Its enrichment and PDGF-BB ligand stimulation of $CD4^+$ T cells showed no evidence of IL-22 expression. Similarly, TIE-1 was only identified on vascular endothelial cells previously (Sato *et al* 1995) and may potentially relate to endothelial cell adhesion and T cell extravasation rather than IL-22 expression.

For these reasons, it remains doubtful whether expression of FGFR2, PDGFR, or TIE1 on T cells is linked to IL-22 expression. The original research that identified these 3 receptors used a library of T helper cell clones derived from the skin of patients with atopic dermatitis (Eyerich *et al* 2009). It is likely that the environment of the skin and the clinical condition of the patients could have accounted for differences between skin Th_{22} cells and peripheral blood Th_{22} cells. It is also possible that the study of only several T cell clones from the skin may not be representative. In conclusion, IL-22^+ T cells could not be obtained either through selective isolation of putative Th_{22}-receptors FGFR2, PDGFR, or TIE-1-positive T cells or through their ligand stimulation. We, therefore, decided to derive T cell lines directly from the bronchial mucosa of asthmatics and obtain individual CD4 T cells through serial dilution and clonal expansion.

2.2. Human asthmatic bronchial mucosa

Each individual T cell line created from asthmatic bronchial mucosal tissue contained IL-22^+ memory T cells. The overwhelming majority of IL-22^+ T cells expressed both the $\alpha\beta$-TCR^+ and $CD4^+$ receptor. While the T cell lines contained mainly $\alpha\beta$-TCR^+ $CD4^+$ T cells, the frequency of IL-22 expression in $\alpha\beta$-TCR^+ and $\gamma\delta$-TCR^+ as well as $CD4^+$ and $CD8^+$ T cells was almost identical. The analysis of IL-22^+ $\alpha\beta$-TCR^+ $CD4^+$ T helper profiles was similar across all asthmatic T cell lines and showed that the majority of IL-22^+ T helper cells co-expressed IFNγ ($Th_{1/22}$: 43.8%). The second largest IL-22^+ T helper cell population expressed only IL-22 (Th_{22}: 22.2%) while the number of cells co-expressing IL-17A was low in all cell lines ($Th_{17/22}$: 10.2%). Co-expression of IL-22 with IL-4 was absent or negligible.

The subsequent isolation and clonal expansion of individual T cells failed to generate enough T cell clones to represent each T helper subset. While Th_1 and Th_2 clones were easily obtained, Th_{22} (and Th_{17}) cells were not. Only three IL-22^+ T cell clones were identified which, in addition, also expressed various levels of IFNγ, IL-17A, and IL-4. Thus, IL-22^+ $CD4^+$ T cells within T cell lines derived from bronchial mucosal tissue belonged largely to the Th_1 and Th_{22} but not Th_{17} or Th_2 subset yet clonal expansion failed to obtain enough T cell clones for a detailed comparative analysis. While the cloning efforts may have eventually yielded enough CD4 T cell clones, the method proved too time consuming and labour intensive to continue.

The study of T cells derived from the asthmatic bronchial mucosa was limited by the availability of clinical samples due to the invasiveness of the procedure and lack of tissue from normal healthy controls. Nonetheless, it proved possible to generate T cell lines from 6 out of 10 biopsies of which 4 were derived from non-allergic asthmatics and 2 from allergic asthmatics. The T cell lines were very similar in composition to other T cell lines showing a similar ratio of CD4/CD8, as well as $\gamma\delta$ T cells (Wilson *et al* 1992, Gratziou *et al* 1992, Crocker *et al* 1998, Krug *et al* 2001). The analysis of IL-22^+ T cells derived from the asthmatic bronchial mucosa was novel. Previous studies assessed the occurrence of IL-22 in asthmatic patients only using peripheral blood (Wong *et al* 2009, Zhu *et al* 2011), where a general increase in IL-22^+ (and IL-17^+) $CD4^+$ T cells correlating modestly with asthma severity was observed. The characterisation of IL-22^+ T cells also included the cytokine IFNγ which, despite its strong link to IL-22 (and IL-17), has been largely overlooked. Its importance has been documented by renewed interest in IL-$17A^+$ IFNγ^+ T cells which are now believed to involved in the pathology of many inflammatory conditions (Annunziato *et al* 2007, Kebir *et al* 2009, Lin *et al* 2009, Boniface *et al* 2010, Cohen *et al* 2011).

The results of this study indicate that the majority of IL-22^+ T cells within the memory T cell pool of the asthmatic lung belonged to the Th_1 subset. The identification of a separate subset of Th_{22} cells *in vivo* had been suggested in several previous studies. Very early on, the observation was made that IL-22^+ and IL-17^+ T cells occur largely as distinct populations (Scriba *et al* 2008) and recent *in vivo* observations support that both cytokines show only minor co-expression (Matthews *et al* 2011, Rudloff *et al* 2012, Zhang *et al* 2012, Cowan *et al* 2012). If IL-22 was released by T cells during allergen provocation within the lung, it must have either been derived from the smaller Th_{22} cell population or released selectively from Th_1 cells. The latter would not be without controversy even though allergen-specific Th_1 cells have been identified in allergic individuals (Oseroff *et al* 2010).

In conclusion, the study of the T cell lines derived from bronchial biopsies from asthmatics yielded novel insights. The majority of IL-22 expressing $CD4^+$ T cells within the T cell lines generated from bronchial tissue were not related to Th_2 or Th_{17} cells but were more likely related to a Th_1 and, possibly, a smaller Th_{22} subset. However, a more detailed study of individual T cell clones of distinct lineage (Th_1, Th_2, Th_{17}, Th_{22}) would have been required to confirm the existence of a distinct Th_{22} cell lineage.

3. IL-22 in human tissue regeneration and repair

3.1. IL-22 Receptor expression in human bronchial epithelial cell lines

In total cell lysates, both IL-22 receptor RA1 and -RA2 were identified to be expressed constitutively by primary bronchial epithelial cells, smooth muscle cells and fibroblast cell lines but not peripheral blood $CD4^+$ T cells. Compared to each other, bronchial epithelial, smooth muscle and fibroblast cells expressed similar levels of IL-22RA1 and -RA2 protein. The detection of IL-22RA1 resulted in a much stronger signal than the detection of IL-22RA2. Surface expression of IL-22RA1 was confirmed on all three cell lines. More than half of all bronchial epithelial cells stained positive for IL-22RA1. In airway smooth muscle cells and fibroblast, IL-22RA1 was only detected on less than 5% of cells. Exposure of bronchial epithelial cells to various T cell cytokines revealed that IL-22RA1 was up regulated by IFNγ. This regulation was dose- and time- dependent and occurred not just in bronchial epithelial cells but also airway smooth muscle cells and fibroblasts. Thus, all three primary structural cell lines had the capacity to express IL-22 receptors while up regulating IL-22RA1 in the presence of IFNγ.

Western blotting was selected to analyse the constitutive expression of both IL-22RA1 and IL-22RA2. Both IL-22RA1 and IL-22RA2 antibodies recognised a specific band corresponding to the correct molecular weight of their target protein and showed no interference in combination with the anti-α-tubulin control. The observed difference in strength between the IL-22RA1 and IL-22RA2 may have related to a quantitative difference. This evaluation has to be viewed with caution as the western blots were not designed to be quantitative and differences in both antibody binding and signal amplification could influence the observed band intensity. Subsequent analysis of IL-22RA1 surface expression was carried out by flow cytometry and circumvented the need for a fractional analysis (e.g. cytosolic, nuclear, membrane) of IL-22RA1 expression by western blot. One limitation was that the use of trypsin/EDTA to detach the adherent cells from the tissue culture dish could not be avoided. Trypsin is able to cleave exposed proteins including surface receptors and could, thus, artificially lower the recorded surface receptor expression. This effect, however,

would have been similar for bronchial epithelial, smooth muscle and fibroblast cells, such that a relative comparison of IL-22RA1 expression between the different cells lines should still be valid.

The regulation of IL-22RA1 and IL-22RA2 was studied using RT-PCR. This technique allowed the detection of small changes in IL-22RA1 and RA2 regulation even in the absence of protein expression and surface translocation. Little is known about IL-22RA2, its time point of expression and regulation. Its expression was, therefore, screened both at 6 and 24 hours using two differently designed probe sets. Both probe sets detected low level IL-22RA2 expression (ct 29-30) which remained unchanged.

The investigation of IL-22RA1 and IL-22RA2 protein expression within the skin reported in this thesis pointed to a wider range of structural cell sources (see Results 1.2.4, page 113). Western blot confirmed that not only bronchial epithelial cells but also fibroblasts and smooth muscle cells were able to express IL-22RA1 and low levels of IL-22RA2 under constitutive conditions. The strong expression of IL-22RA1 on bronchial epithelial cells was in line with previous studies which identified cells of epithelial origin as the main target of IL-22 (Wolk *et al* 2004, Boniface *et al* 2005, Aujla *et al* 2008). Results showed a difference between the IL-22RA1 surface expression on growing and terminally differentiated bronchial epithelial cells. While our cells showed a surface expression of >50%, terminally differentiated cells cultured within an air-liquid-interface showed a surface expression of only 15% (Aujla *et al* 2008). Fibroblasts had been investigated previously showing that synovial, ganglia and myofibroblasts were able to express IL-22RA1 and/or respond to IL-22 in culture (Andoh *et al* 2005, Ikeuchi *et al* 2005, Wolk *et al* 2009). However, these studies have remained largely un-cited and overlooked. Similarly, airway muscle cells have been largely overlooked. The expression of IL-22RA1 in smooth muscle cells has, now, been replicated by others (Zhang *et al* 2011, Chang *et al* 2011) validating the findings reported here. Studies on IL-22RA2 expression are limited. Type-II pneumocytes (alveolar epithelial cells) have been shown to express IL-22RA2 mRNA *in vivo* (Xu *at al* 2001). Investigations of the regulation of IL-22RA1 by IFNγ was reflective of one study carried out *in vitro* using keratinocytes, where both IFNγ and TNFα caused increased IL-22RA1 mRNA up regulation in culture (Wolk *et al* 2005).

The expression of IL-22RA1 on structural cells other than epithelial cells highlight a greater functional diversity of IL-22 beyond “mucosal immunity” alone. The association of fibroblasts, in particular, suggests a potential role in tissue repair processes. Airway smooth muscle cells, for example, have recently been shown to respond to IL-22 with increased migration (Chang *et al* 2011).

The western blot showed that IL-22RA2 was present at low levels in each cell type. The fact that IL-22 did not increase IL-22RA2 mRNA indicated that this basal IL-22RA2 expression is less likely to relate to a regulatory feedback loop within IL-22 target cells. Previously, dual immunochemistry revealed that both IL-22RA1 and IL-22RA2 were expressed on, separate but otherwise, very similar cells within the skin. Both receptors may potentially be expressed by the same cell-type but at different stages of activation or differentiation. The later being supported by the identification of IL-22RA2 in early $HSP47^{+}$ fibroblasts while fully differentiated fibroblast were shown to be IL-$22RA1^{+}$ (Ikeuchi *et al* 2005).The detection of both IL-22RA1 and IL-22RA2 by western blot might, therefore, relate to an artefact of cell culture in which primary cells are maintained in an artificial and not fully differentiated state.

The regulation of IL-22RA1 by IFNγ alone indicates a strong functional connection of IL-22 during Th_1 rather than Th_2-associated inflammation. IFNγ is not just a Th_1 cytokine but is also released by many innate sources very early on during inflammation (e.g. alveolar macrophages). Interestingly, the data showed no further evidence of a Th_1/Th_2 disparity. Th_2-associated cytokines neither down regulated IL-22RA1 expression nor up regulated IL-22RA2. This was reflected *in vivo* where IL-22RA1 and IL-22RA2 expression remained unchanged during experimental allergic inflammation within the skin.

To understand the function role of IL-22, we needed to study the IL-22 target cells in more detail. On the basis of IL-22RA1 surface expression, only primary bronchial epithelial cells appeared suitable to be used as a model to study the function of IL-22 *in vitro*. The low IL-22RA1 surface expression on cultured pulmonary fibroblasts was unexpected, in view of the previous *in vivo* findings in the skin, where IL-22RA1 was abundantly expressed on vimentin-positive early fibroblasts (see Results 1.2, Figure 28 page 118). Based on these results, the study focused exclusively on bronchial epithelial cells and the role of IL-22 during epithelial injury and repair.

3.2. Effect of IL-22 on bronchial epithelial cells

To test our hypothesis that “Interleukin-22 is involved in tissue regeneration and repair”, a functional assay to study the effects of IL-22 on bronchial epithelial wound healing *in vitro* was set up.

3.2.1. Wound healing

The results showed that in an *in vitro* wound healing assay, IL-22 was able to improve epithelial wound closure. This effect occurred in a dose- and time dependent manner and was not influenced by the presence of IL-17A which, on its own, showed no visible effect on

wound closure. Thus, IL-22 may likely augment wound closure following injury, independent of IL-17A expression.

The wound healing assay (Todaro *et al* 1965, Haudenschild *et al* 1979) allows a detailed study of the phenotypic shift of epithelial cells after injury (Kheradmand *et al* 1994, Garat *et al* 1996). These include, in particular, the changes in cell-cell interactions (e.g. tight junctions), the extra-cellular matrix (e.g. MMPs), cytoskeleton arrangements (e.g. actin reorganisation), cell spreading and migration (Kim *et al* 2010). While these events encompass many of the early features of wound closure, the assay cannot address the influence of IL-22 on chemotaxis or cellular proliferation and cannot replicate the complex interactions of several different progenitor, structural and inflammatory cells types during *in vivo* wound healing. The use of primary rather than immortalised bronchial epithelial cells strengthened the study's findings. Primary cells are not commonly selected for functional studies as they are costly and can only be expanded for a short period of time (e.g. passage 4 to 5) hence limiting the number of experiments that can be carried out. However, primary cells retain the majority of functions seen *in vivo* as they are not affected by the side effects of immortalisation and long term culture. Both factors have been linked to alter functional responses in immortalised cell lines leading to sometimes opposing outcomes *in vitro* (Sandberg *et al* 2005, Olsavsky *et al* 2007, Pan *et al* 2009).

The wound healing assay has a simplistic setup but proved difficult to establish and carry out. A major drawback was the great variability of epithelial responses which led to a high inter-assay variation (see Methods 3.2 Figure 13, page 96). The requirement to obtain a homogenous monolayer of epithelial cells was likely the main cause for the highly variable responsiveness of cells *in vitro* as any cell crowding quickly led to cell cycle arrest and cell death *in vitro* (Liang *et al* 2007). Despite this assay variability, the data illustrated a clear and statistically significant effect of IL-22 in enhancing wound closure.

The bronchial epithelium is a relatively new target of IL-22 to be investigated but the results confirmed that the functional association of IL-22 in wound healing is likely a conserved effect which occurs across different cell types and tissues (Boniface *et al* 2005, Brand *et al* 2006, Sa *et al* 2007, Pickert *et al* 2009, Brand *et al* 2007, Eyerich *et al* 2009). This confirmation highlights the need to better understand the role of IL-22 in relation to tissue regeneration and repair within the lung and its potential contribution to asthma. In conclusion, the positive functional response of our human bronchial epithelial cells to IL-22 *in vitro* suggested the need to perform a more detailed functional analysis of the mechanism of IL-22 associated wound healing *in vitro*. As these changes remained poorly defined, the contribution of IL-22 on changes such as cell survival, cell-cell adhesion, extracellular matrix

interactions, cell spreading, migration, and proliferation needed to be addressed. To do so, the subsequent investigation focused first on epithelial proliferation, a feature that could not be addressed by the use of the wound healing assay only.

3.2.2 Proliferation

The results showed that the different cell cycle stages of bronchial epithelial cells remained unaffected by the presence of IL-22 *in vitro*. IL-22 neither decreased the number of resting (G0/1-phase) cells nor increased synthesising (S-phase) and dividing (G2M-phase) cells. As a positive control, bronchial epithelial cells were exposed to TGFβ1, which decreased the percentage of synthesising (S-phase) and increased the number of resting (G0/1-phase) cells. This observation strengthened the finding that IL-22, on its own, showed no effect on bronchial epithelial cell proliferation.

To quantify IL-22 and its effect on cellular proliferation, propidium iodide staining (Krishan 1975) was selected. This method allows the visualisation of the individual cell cycle stages and thus provides more detailed information then a traditional proliferation assay such as [methyl^{3}H]-thymidine incorporation. In comparison to [methyl^{3}H]--thymidine incorporation, propidium iodide staining is not as sensitive but can be carried out without any radioactive safety measures. In addition, propidium iodide is not incorporated into living cells and, thus, does not artificially change the proliferative behaviour of cells *in vitro* (Hu *et al* 2002).

Previous evidence implying an effect of IL-22 on epithelial proliferation had so far been indirect (Brand *et al* 2006, Li *et al* 2009, Ren *et al* 2009).

On the basis of the experimental findings, IL-22 had no direct influence on epithelial cellular proliferation. As cellular proliferation occurs later on during wound healing, I hypothesised that IL-22's function may specifically relate to early events in wound healing. These events potentially include changes in cell-cell adhesion and cell matrix interactions that are often initiated by early pro-inflammatory cytokines such as IFNγ (Crosby and Waters 2010). In conclusion, the effects of IL-22 on enhancing epithelial repair appeared independent of any effect on proliferation. It remains to be established if IL-22 could influence other earlier events during wound closure.

3.2.3 Gene expression

The study showed that IL-22 on its own was able to increase the expression of several known IFNγ associated inflammatory genes. These included the adhesion molecule ICAM1, the cytokine IL-6, and the chemokines CCL2, CXCL9,-10 and -11. While IL-22 alone was a modest inducer of these genes, IL-22 was observed to markedly enhance the effect of IFNγ

on epithelial expression of these genes that included particularly epithelial CXCL10, and CXC11. IFNγ-associated repair genes showed only a modest regulation by IL-22. IL-22 was able to induce both MMP1 and MMP10 but showed no synergy with IFNγ. IFNγ-regulated growth factors BMP2, TYMP, TGFα, INHBA and VEGFc were almost unchanged in the presence of IL-22. IL-22, however, enhanced the IFNγ associated expression. This enhancement was seen for the expression of all growth factor genes and increases in gene expression reached statistical significance for TYMP, BMP2 and TGFα. Thus IL-22, while being a modest inducer of IFNγ-associated genes alone, was able to enhance the effect of IFNγ on many early injury- and repair-associated genes.

Previous studies have addressed mainly IL-22's role in mucosal immunity. While these studies were carried out using IL-22 in combination with IL-17A or TNFα, they made very similar observations. In particular, the enhanced induction of IL-6 (Brand *et al* 2007, Ma *et al* 2008, Sonnenberg *et al* 2010), CCL2, CXCL9, -10, and -11 (Eyerich *et al* 2009, Hosokawa *et al* 2009) had been reported previously in keratinocytes as well as hepatocytes. Only the induction of IL-8/CXCL8, which had been reported in numerous studies (Wolk *et al* 2004, Brand *et al* 2006, 2007, Koga *et al* 2008, Nograles *et al* 2008, Wolk *et al* 2009), could not be observed. Similarly previous research which addressed the function of IL-22 in human bronchial epithelial cells also failed to observe an effect of IL-22 on IL-8/CXCL8 gene expression (Aujla *et al* 2008).

The study of IL-22 and its effect on multiple repair associated genes, on the other hand, was novel. Individually, some previous studies provide support for the identified IL-22-associated induction of MMP1, -10 (Andoh *et al* 2005, Boniface *et al* 2005, Wolk *et al* 2006) and TGFα (Wolk *et al* 2004).

In summary, the results confirmed that IL-22 exhibits a dual role in both amplifying the inflammatory response whilst promoting events related to tissue repair. IL-22 could be considered an early pro-inflammatory cytokine. Yet, its functional role appears to differ from that of other classic pro-inflammatory cytokines (e.g. IFNγ, TNFα and IL-17A). These results as well as previous studies showed that IL-22 on its own was only a weak inducer of effector gene expression. Its effect was only observed in the presence of other pro-inflammatory cytokines. I hypothesise that IL-22, therefore, has a slightly different role and may act more as an immune-modulator than primary effector cytokine. I also hypothesise that IL-22's synergistic effect with pro-inflammatory cytokines such as IFNγ is more selective as it may appear in the small dataset of studied candidate genes. It would, therefore, be interesting to

determine how IL-22 alters the “responsiveness” of structural cells to inflammatory cues and if this could be predictive of its overall function.

In conclusion, the data suggested that IL-22 was linked to both early events in inflammation as well as repair. As IL-22 exhibited a similar function in enhancing the effect of IFNγ, TNFα, and IL-17A, it is probable that IL-22 might, more broadly, target intracellular signalling pathways which, in turn, may enhance the responsiveness of cells to inflammatory cues. The repair related functions remain poorly defined.

In relation to hypothesis

Hypothesis 1

While the evidence suggested a putative role of IL-22 in chronic allergic inflammation, little had been known about IL-22's expression during the allergic reaction itself.

The detection of IL-22, 24 hours after bronchial segmental allergen challenge, in allergic asthmatics was unexpected. It was surprising as it was released in significant quantities to be detectable even in undiluted broncho alveolar lavage alongside IL-5 and in the absence of IFNγ and IL-17A/F. Like the *in vivo* data, the *in vitro* allergen PBMC challenge confirmed the induction of IL-22 together with IL-5 after allergen challenge, independent of IL-17 or IFNγ. The strong increase in IL-22, after bronchial segmental allergen challenge, was almost similar to IL-5 and, thus, may have arisen due to the rapid release of stored cytokine from innate immune cell sources. It was, therefore, important to address if IL-22 was derived from T cell or non-T cell sources. One drawback of this study was that the intradermal allergen challenge, which was specifically setup to address this question, was unable to detect IL-22 within the tissue. We can only presume, based on our *in vivo* an *in vitro* challenge data, that IL-22 may have similarly been expressed during intradermal allergen challenge. If IL-22 was, thus, produced, it would have been detectable at least as a stored cytokine e.g. within granules of innate immune cell sources. Yet, the inability to detect IL-22 pointed away from innate sources towards T cell sources. Thus, we can conclude that IL-22 was expressed during human allergic inflammation yet the identity of the IL-22 producing cells remains to be established.

Hypothesis 2

Our second hypothesis aimed to address if IL-22 was expressed by distinct Th_{22} cells as well as other T cell subsets (Th_1, Th_2, Th_{17}). The experimental results indicated that IL-22 was not universally expressed across different subsets. While IL-22 expression occurred within Th_2 driven inflammation, Th_2 cells themselves showed no ability to express IL-22. Our data highlighted the strong relationship between IL-22 and Th_1 cells but confirmed the divergence between IL-22 and IL-17 expressing cells. In addition, a distinct population of cells reminiscent of the Th_{22} subset could be identified. A major focus of this PhD, therefore, was to address the development of Th_{22} cells, their transcriptional regulation and relationship to other T helper cell lineages. The inability to either differentiate Th_{22} cells *in vitro* or isolate existing Th_{22} cells from peripheral blood or lung tissue *ex vivo* was a major hurdle. It meant that we could not further address if these IL-22^+ $CD4^+$ T cells represented a distinct T cell lineage. We can, therefore, only conclude that IL-22 is a Th_1-related cytokine expressed by Th_1 as well as cells reminiscent of the proposed Th_{22} cell subset.

Hypothesis 3

The identification of a wide range of IL-22RA1$^+$ cells within the skin was novel and indicated that cells of epithelial origin represented not the only target of IL-22 within the tissue. The strong association with the connective tissue, in particular, which is rich in early progenitors, stem cells and fibroblasts, supported the hypothesis that IL-22 had an important role in influencing tissue regenerative and repair processes. While the exact identify of all target cells within the connective tissue could not be specified, it became apparent that fibroblasts were likely one of the cell populations.

Studying epithelial injury and repair *in vivo* shows that sub epithelial early progenitor cell populations play a key role in re-constituting the damaged areas temporarily as well as permanently. Inflammatory cues such as IL-22 may, thus, potentially serve as activation signal for cells to undergo differentiation and maturation. Yet, we are only beginning to develop the right culture techniques to maintain isolated stem cells and early progenitor cells in culture to test these hypotheses in more detail.

The important role of IL-22 on cells of fibroblast origin must have been evident before, as three separate research groups aimed to address the functional response of IL-22 on them *in vitro* (Ikeuchi *et al* 2005, Andoh *et al* 2005, Wolk *et al* 2009). However, like our pulmonary fibroblast, the differentiation and maturation stage of these cells in culture only showed low IL-22RA1 surface expression. It was clear that normal cell culture techniques would not allow a proper functional analysis *in vitro*. The lack of functional response in the previous studies was likely an important factor while they remained largely overlooked and not cited until now. I can only hypothesise about the functional relevance of IL-22 in fibroblasts. From the insight gained studying the functional response of IL-22 in bronchial epithelial cells, it is likely that IL-22 plays a role in early activation and differentiation rather than later processes such as TGFβ1-driven collagen synthesis and deposition.

Another important aspect highlighted was that epithelial cells and underlying early progenitor cells were not the only target of IL-22. Also very different structural cells such as smooth muscle cells showed to be IL-22RA1 positive in culture. Again, due to their low surface expression *in vitro,* the functional response of these cells to IL-22 was not investigated further. However, two recent publications have validated the study's findings and confirmed that cultured smooth muscle cells express IL-22RA1 and responded to IL-22 with increased migration (Chang *et al* 2011). It is, therefore, not unimaginable that IL-22 has a more global effect on multiple structural cells within the tissue in relation to repair. Yet, it is also possible that growing and not fully-matured epithelial cells, mesenchymal cells and smooth muscle

cells share certain functional similarities in culture, allowing them to respond to IL-22 in similar manner. A comparison of cultured bronchial epithelial cells alone showed that growing cells exhibited a >50% IL-22RA1 surface expression (see Figure 42, page 141) while fully-matured cells in an air-liquid-interface culture showed only a 16% surface expression (Aujla *et al* 2008). This also suggests that even bronchial epithelial cells are more likely to respond to IL-22 when they are not as terminally differentiated.

The regulation of IL-22RA1 in bronchial epithelial cells but also fibroblasts and smooth muscle cells was controlled by IFNγ. This observation indicated that the time window of IL-22 expression occurred likely at the onset or immediately after the first inflammatory response. Similarly, the measurement of IL-22RA2 in the bronchoalveolar lavage supported the view that IL-22 function occurred likely during the early phase and was presumably actively blocked during resolution. The investigations into the underlying molecular mechanisms of bronchial epithelial wound healing also indicated that IL-22 had a role in early repair. At least, on its own, IL-22 was unable to influence cellular proliferation. Cellular proliferation is an important feature in wound healing which occurs after an initial re-organisation of the tissue (e.g. extracellular matrix break down, cell de-differentiation, cell spreading). IL-22's main role is, thus, likely related to early protective and regenerative processes within tissue. The gene expression screen, while set up with a different aim, supported this view as IL-22 was able to independently regulate matrix-metalloproteinases such as MMP1 and MMP10. While the study was unable to further address IL-22's influence on these early events, it is likely that the presence of IL-22 may have an influence on cell survival, tight junction break down, cell-matrix degradation, de-differentiation and cellular migration.

Thus, I hypothesise that IL-22 is an early pro-inflammatory cytokine initiating early events in wound repair. To do so, IL-22 targets early progenitor cells within the underlying connective tissue as well as influence damaged structural cells within the tissue to survive, de-differentiate and migrate.

Relevance of IL-22 in allergy and asthma

Our interest in IL-22 in allergy and asthma was formed when studies highlighted a significant increase in IL-22 and Th_{22} cells in atopic dermatitis. These measurements were made during the chronic inflammatory phase of the disorder. Similarly in allergic asthma, evidence suggested that during chronic inflammation, the T helper cell response may become more diverse and could potentially include IL-22 and IL-17 responses.

The now identified expression of the IL-22 expression during allergic inflammation remains difficult to judge with the limited knowledge available. IL-22 was released in a Th_2-driven

environment yet Th_2 cells were unable to express the cytokine. Furthermore, IL-22 showed a strong relationship to the cytokine IFNγ and was not only released by responding atopic but also by responding non-atopic cells in culture. We, therefore, hypothesise that IL-22 may not belong to the adaptive Th_2 response as such.

The release of IL-22 may be a response related to immune cell activation and inflammation itself and, thus, occur before or in parallel with the ensuing Th_2 response. The role of IL-22 specifically in allergy may serve a similar protective role during general inflammation regardless of the bias of the ensuing adaptive immune response. The view that IL-22 may modulate inflammation was supported by recent studies. In these, the authors blocked IL-22 expression during an experimental allergen challenge in mice. This led to an enhanced allergic inflammation and as others had shown to increased cellular damage (Besnard *et al* 2011). This regulation of the allergic immune response by IL-22 was hypothesised to involve IL-10 associated mechanisms augmenting eosinophilic infiltration (Nakagome *at al* 2011). Thus, I believe that IL-22 is an integral part of the immune response protecting the underlying tissue through modulating the severity of inflammation as well as initiating protective and regenerative processes within the tissue

The expression of IL-22 may, thus, be beneficial in asthma. Here, more so than in other atopic conditions, the function and integrity of structural cells within the lung is of particular importance. IL-22 may similarly dampen the severity of allergic inflammation (as recently shown in mice) but maybe, more importantly, improve epithelial function and integrity. Yet, the asthmatic epithelium is altered and itself plays a significant role in the development of asthma and progression of tissue remodelling. Even in the absence of Th_2-driven inflammation, the asthmatic epithelium displays a defective stress and anti-oxidant handling (Sackesen *et al* 2008, Yang *et al* 2009, Schultz *et al* 2010), heightened caspase activity (Truong-tran *et al* 2003, Kuwano *et al* 2007), altered tight junction formation (de Boer *et al* 2008, Holgate *et al* 2009), defective migration and proliferation (Kicic *et al* 2010). Therefore, it remains to be established if IL-22 signalling could be affected in an altered asthmatic epithelium, (e.g. altered IFNλ signalling; Bosco *et al* 2010), or if it could function normally and overcome the inherited alterations in epithelial function.

One feature implicated during this PhD research was IL-22's putative role on mesenchymal progenitor cells and fibroblasts. Mesenchymal-derived fibroblast precursors are normally responsible for tissue repair (Morishima *et al* 2001). In asthma, basal lamina thickening and subepithelial fibrosis are a hallmark of the pathology (Brewster e*t al* 1990, Zhang *et al* 1994). These remodelling processes are unlikely controlled directly by IL-22. Yet IL-22's expression early on during inflammation may lay the ground for precursor activation and differentiation.

Therefore, it has to be carefully judged if the influence of IL-22 may always be positive. It could be possible that repair responses coordinated by IL-22, which may otherwise be protective in normal inflammatory settings, in asthma, may not always lead to a positive outcome.

We are, therefore, only at the beginning of gaining insight into the role of IL-22 in human allergy and asthma. First, we need to better understand IL-22's normal role during inflammation. Only then, could we specifically address its functionality in the altered asthmatic lung.

Conclusion

The investigation showed that IL-22 increased *in vivo* after bronchial segmental allergen challenge in bronchoalveolar lavage obtained from asthmatics but not in control individuals. While its expression following intradermal allergen challenge remained inconclusive, the allergen-specific stimulation of PBMCs *in vitro* confirmed up regulation of IL-22. Yet, IL-22 was observed to increase in PBMC cultures derived not only from atopics but also control subjects.

While the identify of cellular source of IL-22 production *in vivo* has yet to be determined, allergen stimulation of T cells *in vitro* provided evidence suggesting that IL-22 may not have been released by Th_2 cells. Instead, the cytokine profile of IL-22^+ T cells derived, either following allergen-specific expansion *in vitro* or from asthmatic bronchial tissue *ex vivo,* was more reminiscent of cells belonging to the Th_1 and the newly proposed Th_{22} subset.

Structural cells within the tissue appeared to be the major target for IL-22. In the skin, there was wide expression of both IL-22RA1 and IL-22RA2 that co-localised to vimentin, a marker of cells of mesenchymal origin. Further analysis revealed partial co-localisation of IL-22RA2 with HSP-47, a marker of early fibroblasts. Similarly, primary cell lines derived from bronchial tissue (bronchial epithelial cells, smooth muscle cells, pulmonary fibroblasts) showed constitutive expression of both IL-22RA1 and IL-22RA2. While Th_2 cytokines were unable to alter IL-22 receptor expression on these cell lines, the Th_1 cytokine, IFNγ, increased IL-22RA1 mRNA in a dose- and time-dependent manner.

Having identified particularly strong expression of the IL-22 receptor on epithelial cells, primary human bronchial epithelial cells were used to study the function of IL-22. IL-22 synergised with IFNγ in the induction of pro-inflammatory genes (IL-6, CXCL9,-10, and -11) in epithelial cells. On its own, IL-22 was able to augment the healing, *in vitro,* of an injured bronchial epithelial cell layer. While these effects were observed independently of an effect of IL-22 on proliferation, the precise mechanism remains to be determined.

In conclusion, these studies in man show that IL-22 is released after allergen encounter and targets primarily structural as well as progenitor cells (e.g. early fibroblasts) within the tissue. While it is expressed during Th_2-driven inflammation, IL-22, itself, appeared to be strongly a Th_1-related cytokine. The function of IL-22 was related to both inflammation (associated with IFNγ) and repair. Release of IL-22 during the allergic response could relate to its tissue protective functions thus potentially dampening Th_2-driven inflammation. All in all, IL-22

appears to be a Th_1-related cytokine that targets structural cells and progenitor cells, including early fibroblasts, within the tissue. Taken together, these studies suggest that IL-22 has a dual role in early inflammation and repair.

Overview:

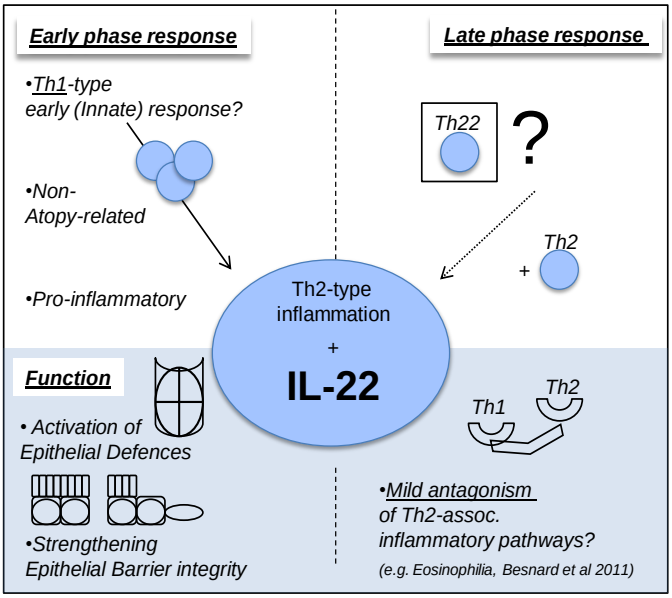


Diagram 5. Potential expression and function of IL-22 during allergic inflammation

Future Outlook

The data generated during the PhD represents the first investigation of IL-22 in human allergy and asthma. The increase in IL-22 expression after allergen challenge in the bronchoalveolar lavage of allergic asthmatics but not healthy controls is therefore an important observation.

Due to the very small sample cohort of 15 allergic asthmatics and 3 healthy controls, this important observation requires validation. To substantiate the result, the study cohort would normally be increased. However, at this point in time, not enough insight into the cytokines function and link to the clinical condition has been gained to justify the additional recruitment of allergic asthmatics.

An alternative would be to re-analyse the samples obtained after intradermal allergen challenge. We now know that the original approach of detecting IL-22 by immuno-fluorescence was flawed. The successful application of in-situ hybridisation could not only achieve the original objective but also shed more light on the cellular source of IL-22.

The original PhD has focused primarily on the role of IL-22 in bronchial epithelial wound healing. If the evidence of IL-22 expression during allergic inflammation becomes more substantial, the influence of the cytokine on other mediators of allergic inflammation needs to be investigated.

Future steps include:

1. Validation of the observed expression of IL-22 in allergy and asthma

2. Clarification if IL-22 is indeed expressed by T cells during the late phase response

3. Investigation if the presence of IL-22 alters the functional response of other cytokines (e.g. IL-4/IL-13) in relation to the bronchial epithelium.

References

Abbal C, Persi L, Bousquet J, Yssel H, Pène J.(1998).Effect of bronchial allergen challenge on in vitro cytokine release by peripheral blood mononuclear cells of atopic patients. Allergy.;53(10):945-50

Abbas AK, Murphy KM, Sher A.(1996).Functional diversity of helper T lymphocytes.Nature. 1996 Oct 31;383(6603):787-93

Abramson MJ, Puy RM, Weiner JM.(2003)Allergen immunotherapy for asthma.Cochrane Database Syst Rev. (4):CD001186.

Abramson MJ, Puy RM, Weiner JM.(2010)Injection allergen immunotherapy for asthma.Cochrane Database Syst Rev.;(8):CD001186

Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F.(2007). Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. Nat Immunol.;8(9):942-9

Afkarian M, Sedy JR, Yang J, Jacobson NG, Cereb N, Yang SY, Murphy TL, Murphy KM.(2002). T-bet is a STAT1-induced regulator of IL-12R expression in naïve CD4+ T cells. Nat Immunol.;3(6):549-57

Aggarwal S, Xie MH, Maruoka M, Foster J, Gurney AL. (2001) Acinar cells of the pancreas are a target of interleukin-22. J Interferon Cytokine Res.;21(12):1047-53

Alam MS, Maekawa Y, Kitamura A, Tanigaki K, Yoshimoto T, Kishihara K, Yasutomo K.(2010). Notch signaling drives IL-22 secretion in CD4+ T cells by stimulating the aryl hydrocarbon receptor. Proc Natl Acad Sci U S A.;107(13):5943-8

Ali FR, Oldfield WL, Higashi N, Larche M, Kay AB (2004) Late asthmatic reactions induced by inhalation of allergen-derived T cell peptides. Am J Respir Crit Care Med, 169; pp. 20–26

Ali FR, Kay AB, Larché M.(2007). Airway hyperresponsiveness and bronchial mucosal inflammation in T cell peptide-induced asthmatic reactions in atopic subjects.Thorax.;62(9):750-57

Amishima M, Munakata M, Nasuhara Y, Sato A, Takahashi T, Homma Y, Kawakami Y.(1998). Expression of epidermal growth factor and epidermal growth factor receptor immunoreactivity in the asthmatic human airway.Am J Respir Crit Care Med.;157(6 Pt 1):1907-12

Anderson GP, (2008) Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet.;372(9643):1107-19

Andoh A, Zhang Z, Inatomi O, Fujino S, Deguchi Y, Araki Y, Tsujikawa T, Kitoh K, Kim-Mitsuyama S, Takayanagi A, Shimizu N, Fujiyama Y. (2005). Interleukin-22, a member of the IL-10 subfamily, induces inflammatory responses in colonic subepithelial myofibroblasts. Gastroenterology ;129(3):969-84.

Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B, Parente E, Filì L, Ferri S, Frosali F, Giudici F, Romagnani P, Parronchi P, Tonelli F, Maggi E, Romagnani S.(2007) Phenotypic and functional features of human Th17 cells.J Exp Med.;204(8):1849-61

Apetoh L, Quintana FJ, Pot C, Joller N, Xiao S, Kumar D, Burns EJ, Sherr DH, Weiner HL, Kuchroo VK.(2010). The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. Nat Immunol.;11(9):854-61

Arora PD, McCulloch CA.(1994). Dependence of collagen remodelling on alpha-smooth muscle actin expression by fibroblasts.J Cell Physiol.;159(1):161-75

Arvidsson MB, Löwhagen O, Rak S.(2007) Early and late phase asthmatic response in lower airways of cat-allergic asthmatic patients--a comparison between experimental and environmental allergen challenge. Allergy.;62(5):488-94

Ashton-Rickardt PG, Tonegawa S.(1994)A differential-avidity model for T-cell selection.Immunol Today. ;15(8):362-6

Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, Reinhart TA, McAllister F, Edeal J, Gaus K, Husain S, Kreindler JL, Dubin PJ, Pilewski JM, Myerburg MM, Mason CA, Iwakura Y, Kolls JK.(2008). IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nat Med.;14(3):275-81.

Awasthi A, Riol-Blanco L, Jäger A, Korn T, Pot C, Galileos G, Bettelli E, Kuchroo VK, Oukka M.(2009). Cutting edge: IL-23 receptor gfp reporter mice reveal distinct populations of IL-17-producing cells.J Immunol.;182(10):5904-8

Baba N, Rubio M, Sarfati M.(2010).Interplay between CD45RA+ regulatory T cells and TNF-alpha in the regulation of human Th17 differentiation. Int Immunol.;22(4):237-44

Baigelman W, Chodosh S.(1977), Bronchodilator action of the anticholinergic drug, ipratropium bromide (Sch 1000), as an aerosol in chronic bronchitis and asthma.Chest.;71(3):324-8.
Barczyk A, Pierzchala W, Sozañska E.(2003).Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine.Respir Med. ;97(6):726-33

Bard JD, Gelebart P, Anand M, Amin HM, Lai R. (2008) Aberrant expression of IL-22 receptor 1 and autocrine IL-22 stimulation contribute to tumorigenicity in ALK+ anaplastic large cell lymphoma. Leukemia.;22(8):1595-603

Barlow JL, Flynn RJ, Ballantyne SJ, McKenzie AN.(2011).Reciprocal expression of IL-25 and IL-17A is important for allergic airways hyperreactivity. Clin Exp Allergy.;41(10):1447-55

Barnes KC, Neely JD, Duffy DL, Freidhoff LR, Breazeale DR, Schou C, Naidu RP, Levett PN, Renault B, Kucherlapati R, Iozzino S, Ehrlich E, Beaty TH, Marsh DG. (1996) Linkage of asthma and total serum IgE concentration to markers on chromosome 12q: evidence from Afro-Caribbean and Caucasian populations. Genomics.;37(1):41-50

Bauchau V, Durham SR.(2004). Prevalence and rate of diagnosis of allergic rhinitis in Europe. Eur Respir J.;24(5):758-64

Bautista JL, Lio CW, Lathrop SK, Forbush K, Liang Y, Luo J, Rudensky AY, Hsieh CS.(2009). Intraclonal competition limits the fate determination of regulatory T cells in the thymus. Nat Immunol.;10(6):610-7

Bayram H, Rusznak C, Khair OA, Sapsford RJ, Abdelaziz MM.(2002). Effect of ozone and nitrogen dioxide on the permeability of bronchial epithelial cell cultures of non-asthmatic and asthmatic subjects.Clin Exp Allergy.;32(9):1285-92

Bentley AM, Meng Q, Robinson DS, Hamid Q, Kay AB, Durham SR.(1993). Increases in activated T lymphocytes, eosinophils, and cytokine mRNA expression for interleukin-5 and granulocyte/macrophage colony-stimulating factor in bronchial biopsies after allergen

inhalation challenge in atopic asthmatics. Am J Respir Cell Mol Biol.;8(1):35-42

Besnard AG, Sabat R, Dumoutier L, Renauld JC, Willart M, Lambrecht B, Teixeira MM, Charron S, Fick L, Erard F, Warszawska K, Wolk K, Quesniaux V, Ryffel B, Togbe D. (2011) Dual Role of IL-22 in allergic airway inflammation and its cross-talk with IL-17A. Am J Respir Crit Care Med.;183(9):1153-63. Epub 2011 Feb 4.

Bisgaard H, Hermansen MN, Loland L, Halkjaer LB, Buchvald F.(2006). Intermittent inhaled corticosteroids in infants with episodic wheezing. N Engl J Med.;354(19):1998-2005

Boniface K, Bernard FX, Garcia M, Gurney AL, Lecron JC, Morel F.(2005). IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes.J Immunol.;174(6):3695-702

Boniface K, Blumenschein WM, Brovont-Porth K, McGeachy MJ, Basham B, Desai B, Pierce R, McClanahan TK, Sadekova S, de Waal Malefyt R.(2010). Human Th17 cells comprise heterogeneous subsets including IFN-gamma-producing cells with distinct properties from the Th1 lineage.J Immunol.;185(1):679-87.

Bosco A, McKenna KL, Firth MJ, Sly PD, Holt PG. (2009).A network modeling approach to analysis of the Th2 memory responses underlying human atopic disease. J Immunol. 15;182(10):6011-21

Bosco A, Ehteshami S, Stern DA, Martinez FD.(2010). Decreased activation of inflammatory networks during acute asthma exacerbations is associated with chronic airflow obstruction.Mucosal Immunol.;3(4):399-409

Böttcher MF, Bjurström J, Mai XM, Nilsson L, Jenmalm MC.(2003).Allergen-induced cytokine secretion in atopic and non-atopic asthmatic children. Pediatr Allergy Immunol.;14(5):345-50.

Boushey HA, Sorkness CA, King TS, Sullivan SD, Fahy JV, Lazarus SC, Chinchilli VM, Craig TJ, Dimango EA, Deykin A, Fagan JK, Fish JE, Ford JG, Kraft M, Lemanske RF Jr, Leone FT, Martin RJ, Mauger EA, Pesola GR, Peters SP, Rollings NJ, Szefler SJ, Wechsler ME, Israel E; National Heart, Lung, and Blood Institute's Asthma Clinical Research Network.(2005). Daily versus as-needed corticosteroids for mild persistent asthma.N Engl J Med.;352(15):1519-28

Bradding P, Feather IH, Howarth PH, Mueller R, Roberts JA, Britten K, Bews JP, Hunt TC, Okayama Y, Heusser CH, et al.(1992). Interleukin 4 is localized to and released by human mast cells. J Exp Med.;176(5):1381-6

Brand S, Beigel F, Olszak T, Zitzmann K, Eichhorst ST, Otte JM, Diepolder H, Marquardt A, Jagla W, Popp A, Leclair S, Herrmann K, Seiderer J, Ochsenkühn T, Göke B, Auernhammer CJ, Dambacher J.(2006). IL-22 is increased in active Crohn's disease and promotes proinflammatory gene expression and intestinal epithelial cell migration. Am J Physiol Gastrointest Liver Physiol.;290(4):G827-38

Brand S, Dambacher J, Beigel F, Zitzmann K, Heeg MH, Weiss TS, Prüfer T, Olszak T, Steib CJ, Storr M, Göke B, Diepolder H, Bilzer M, Thasler WE, Auernhammer CJ. (2007) IL-22-mediated liver cell regeneration is abrogated by SOCS-1/3 overexpression in vitro. Am J Physiol Gastrointest Liver Physiol.;292(4):G1019-28.

Bresciani M, Parisi C, Menghi G, Bonini S.(2005). The hygiene hypothesis: does it function worldwide?Curr Opin Allergy Clin Immunol.;5(2):147-51

Brewster CE, Howarth PH, Djukanovic R, Wilson J, Holgate ST, Roche WR.(1990). Myofibroblasts and subepithelial fibrosis in bronchial asthma. Am J Respir Cell Mol Biol.;3(5):507-11.

Brüstle A, Heink S, Huber M, Rosenplänter C, Stadelmann C, Yu P, Arpaia E, Mak TW, Kamradt T, Lohoff M.(2007). The development of inflammatory T(H)-17 cells requires interferon-regulatory factor 4. Nat Immunol.;8(9):958-66

Bucchieri F, Puddicombe SM, Lordan JL, Richter A, Buchanan D, Wilson SJ, Ward J, Zummo G, Howarth PH, Djukanović R, Holgate ST, Davies DE.(2002). Asthmatic bronchial epithelium is more susceptible to oxidant-induced apoptosis. Am J Respir Cell Mol Biol.;27(2):179-85

Bullens DM, De Swerdt A, Dilissen E, Kasran A, Kroczek RA, Cadot P, Casaer P, Ceuppens JL.(2005). House dust mite-specific T cells in healthy non-atopic children.Clin Exp Allergy.;35(12):1535-41

Burchill MA, Yang J, Vogtenhuber C, Blazar BR, Farrar MA.(2007). IL-2 receptor beta-dependent STAT5 activation is required for the development of Foxp3+ regulatory T cells.J Immunol.;178(1):280-90

Bureau JF, Montagutelli X, Bihl F, Lefebvre S, Guénet JL, Brahic M. (1993) Mapping loci influencing the persistence of Theiler's virus in the murine central nervous system. Nat Genet.;5(1):87-91

Busse WW.(2012) The brain and asthma: what are the linkages?. Chem Immunol Allergy.;98:14-31.

Byrd VM, Kilkenny DM, Dikov MM, Reich MB, Rocheleau JV, Armistead WJ, Thomas JW, Miller GG.(2003). Fibroblast growth factor receptor-1 interacts with the T-cell receptor signalling pathway.Immunol Cell Biol.;81(6):440-50

Carroll KN, Wu P, Gebretsadik T, Griffin MR, Dupont WD, Mitchel EF, Hartert TV.(2009). Season of infant bronchiolitis and estimates of subsequent risk and burden of early childhood asthma.J Allergy Clin Immunol.;123(4):964-6

Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE. (1982). Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. J Allergy Clin Immunol. ;70(3):170-7

Castellani ML, Felaco P, Galzio RJ, Tripodi D, Toniato E, De Lutiis MA, Fulcheri M, Caraffa A, Antinolfi P, Tetè S, Felaco M, Conti F, Pandolfi F, Theoharides TC, Shaik-Dasthagirisaheb YB.(2010)IL-31 a Th2 cytokine involved in immunity and inflammation.Int J Immunopathol Pharmacol. ;23(3):709-13

Cella M, Fuchs A, Vermi W, Facchetti F, Otero K, Lennerz JK, Doherty JM, Mills JC, Colonna M.(2009). A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity.Nature.;457(7230):722-5

Cembrzynska-Nowak M, Szklarz E, Inglot AD, Teodorczyk-Injeyan JA.(1993).Elevated release of tumor necrosis factor-alpha and interferon-gamma by bronchoalveolar leukocytes from patients with bronchial asthma. Am Rev Respir Dis. ;147(2):291-5

Chakir J, Shannon J, Molet S, Fukakusa M, Elias J, Laviolette M, Boulet LP, Hamid Q.(2003).Airway remodeling-associated mediators in moderate to severe asthma: effect of

steroids on TGF-beta, IL-11, IL-17, and type I and type III collagen expression. J Allergy Clin Immunol.;111(6):1293-8

Chanez P, Bourdin A, Vachier I, Godard P, Bousquet J, Vignola AM.(2004). Effects of inhaled corticosteroids on pathology in asthma and chronic obstructive pulmonary disease.Proc Am Thorac Soc.;1(3):184-90

Chang Y, Al-Alwan L, Risse PA, Roussel L, Rousseau S, Halayko AJ, Martin JG, Hamid Q, Eidelman DH.(2011). TH17 cytokines induce human airway smooth muscle cell migration. J Allergy Clin Immunol.;127(4):1046-53.e1-2

Charlesworth EN, Kagey-Sobotka A, Schleimer RP, Norman PS, Lichtenstein LM. (1991) Prednisone inhibits the appearance of inflammatory mediators and the influx of eosinophils and basophils associated with the cutaneous late-phase response to allergen. J Immunol.;146(2):671-6

Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL.(1994). Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. Science.;265(5176):1237-40

Chen Z, Laurence A, Kanno Y, Pacher-Zavisin M, Zhu BM, Tato C, Yoshimura A, Hennighausen L, O'Shea JJ.(2006). Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells.Proc Natl Acad Sci U S A.;103(21):8137-42

Cheng F, Guo Z, Xu H, Yan D, Li Q. (2009) Decreased plasma IL22 levels, but not increased IL17 and IL23 levels, correlate with disease activity in patients with systemic lupus erythematosus. Ann Rheum Dis.;68(4):604-6

Cho SH, Stanciu LA, Holgate ST, Johnston SL.(2005).Increased interleukin-4, interleukin-5, and interferon-gamma in airway CD4+ and CD8+ T cells in atopic asthma.Am J Respir Crit Care Med.;171(3):224-30

Choe MM, Sporn PH, Swartz MA.(2006). Extracellular matrix remodeling by dynamic strain in a three-dimensional tissue-engineered human airway wall model. Am J Respir Cell Mol Biol.;35(3):306-13

Chung Y, Yang X, Chang SH, Ma L, Tian Q, Dong C.(2006). Expression and regulation of IL-22 in the IL-17-producing CD4+ T lymphocytes.Cell Res.;16(11):902-7

Clark (2007) Anti-inflammatory functions of glucocorticoid-induced genes, Mol and Cell Endocrinol; V.275,1–2, 15 p79–97

Cockcroft DW, Murdock KY, Kirby J, Hargreave F.(1987)Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine.Am Rev Respir Dis.;135(1):264-7

Cohen RT, Canino GJ, Bird HR, Shen S, Rosner BA, Celedón JC.(2007). Area of residence, birthplace, and asthma in Puerto Rican children.Chest.;131(5):1331-8

Cohen CJ, Crome SQ, MacDonald KG, Dai EL, Mager DL, Levings MK.(2011). Human Th1 and Th17 cells exhibit epigenetic stability at signature cytokine and transcription factor loci.J Immunol.;187(11):5615-26

Connett GJ.(2000). Bronchoalveolar lavage. Paediatr Respir Rev. ;1(1):52-6.

Conti HR, Shen F, Nayyar N, Stocum E, Sun JN, Lindemann MJ, Ho AW, Hai JH, Yu JJ, Jung JW, Filler SG, Masso-Welch P, Edgerton M, Gaffen SL. (2009) Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. J Exp Med. ;206(2):299-311

Cookson WO, Hopkin JM.(1988). Dominant inheritance of atopic immunoglobulin-E responsiveness. Lancet. 1988 Jan 16;1(8577):86-8

Cookson W.(2004). The immunogenetics of asthma and eczema: a new focus on the epithelium.Nat Rev Immunol.;4(12):978-88

Corren J. (1999).The relationship between allergic rhinitis and bronchial asthma. Curr Opin Pulm Med. ;5(1):35-7.

Cosentini R, Tarsia P, Canetta C, Graziadei G, Brambilla AM, Aliberti S, Pappalettera M, Tantardini F, Blasi F.(2008). Severe asthma exacerbation: role of acute Chlamydophila pneumoniae and Mycoplasma pneumoniae infection. Respir Res.;9:48

Cosmi L, Annunziato F, Maggi E, Romagnani S, Manetti R.(2001) Chemoattractant receptors expressed on type 2 T cells and their role in disease.Int Arch Allergy Immunol.;125(4):273-9

Cosmi L, Maggi L, Santarlasci V, Capone M, Cardilicchia E, Frosali F, Querci V, Angeli R, Matucci A, Fambrini M, Liotta F, Parronchi P, Maggi E, Romagnani S, Annunziato F. (2010) Identification of a novel subset of human circulating memory CD4(+) T cells that produce both IL-17A and IL-4. J Allergy Clin Immunol.;125(1):222-30.e1-4

Cousins DJ, Lee TH, Staynov DZ.(2002).Cytokine coexpression during human Th1/Th2 cell differentiation: direct evidence for coordinated expression of Th2 cytokines.J Immunol.;169(5):2498-506

Coutinho AE, Chapman KE.(2011)The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Mol Cell Endocrinol. ;335(1):2-13.

Cowan J, Pandey S, Filion LG, Angel JB, Kumar A, Cameron DW.(2012). Comparison of interferon-γ-, interleukin (IL)-17- and IL-22-expressing CD4 T cells, IL-22-expressing granulocytes and proinflammatory cytokines during latent and active tuberculosis infection. Clin Exp Immunol.;167(2):317-29. doi: 10.1111/j.1365-2249.2011.04520.x

Crellin NK, Trifari S, Kaplan CD, Cupedo T, Spits H.(2010). Human NKp44+IL-22+ cells and LTi-like cells constitute a stable RORC+ lineage distinct from conventional natural killer cells. J Exp Med.;207(2):281-90

Crocker IC, Gupta K, Townley RG, Khan MM.(1998). The profile of the cytokines secreted during the generation of T-helper cells from atopic asthmatic subjects.J Asthma.;35(2):187-201

Crosby LM, Waters CM.(2010). Epithelial repair mechanisms in the lung.Am J Physiol Lung Cell Mol Physiol.;298(6):L715-31.

Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, Lucian L, To W, Kwan S, Churakova T, Zurawski S, Wiekowski M, Lira SA, Gorman D, Kastelein RA, Sedgwick JD.(2003). Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature.;421(6924):744-8

Cupedo T, Crellin NK, Papazian N, Rombouts EJ, Weijer K, Grogan JL, Fibbe WE, Cornelissen JJ, Spits H.(2009). Human fetal lymphoid tissue-inducer cells are interleukin 17-producing precursors to RORC+ CD127+ natural killer-like cells. Nat Immunol.;10(1):66-74

Daly JW, Padgett WL, Shamim MT.(1986) Analogues of caffeine and theophylline: effect of structural alterations on affinity at adenosine receptors.J Med Chem.;29(7):1305-8.

Dambacher J, Beigel F, Zitzmann K, De Toni EN, Göke B, Diepolder HM, Auernhammer CJ, Brand S.(2009). The role of the novel Th17 cytokine IL-26 in intestinal inflammation.Gut.;58(9):1207-17

Dambacher J, Beigel F, Zitzmann K, Heeg MH, Göke B, Diepolder HM, Auernhammer CJ, Brand S. (2008) The role of interleukin-22 in hepatitis C virus infection. Cytokine.;41(3):209-16
Davidson TS, DiPaolo RJ, Andersson J, Shevach EM.(2007). Cutting Edge: IL-2 is essential for TGF-beta-mediated induction of Foxp3+ T regulatory cells.J Immunol.;178(7):4022-6

de Boer WI, Sharma HS, Baelemans SM, Hoogsteden HC, Lambrecht BN, Braunstahl GJ.(2008). Altered expression of epithelial junctional proteins in atopic asthma: possible role in inflammation. Can J Physiol Pharmacol.;86(3):105-12

de Bruin-Weller MS, Weller FR, De Monchy JG. (1999) Repeated allergen challenge as a new research model for studying allergic reactions. Clin Exp Allergy. 29(2):159-65

de Luca A, Zelante T, D'Angelo C, Zagarella S, Fallarino F, Spreca A, Iannitti RG, Bonifazi P, Renauld JC, Bistoni F, Puccetti P, Romani L.(2010). IL-22 defines a novel immune pathway of antifungal resistance. Mucosal Immunol.;3(4):361-73

de Monchy JG, Kauffman HF, Venge P, Koëter GH, Jansen HM, Sluiter HJ, De Vries K.(1985) Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. Am Rev Respir Dis.;131(3):373-6.

de Moura PR, Watanabe L, Bleicher L, Colau D, Dumoutier L, Lemaire MM, Renauld JC, Polikarpov I. (2009) Crystal structure of a soluble decoy receptor IL-22BP bound to interleukin-22. FEBS Lett.;583(7):1072-7

de Wit J, Souwer Y, van Beelen AJ, de Groot R, Muller FJ, Klaasse Bos H, Jorritsma T, Kapsenberg ML, de Jong EC, van Ham SM.(2011). CD5 costimulation induces stable Th17 development by promoting IL-23R expression and sustained STAT3 activation. Blood.;118(23):6107-14

Dhiman R, Indramohan M, Barnes PF, Nayak RC, Paidipally P, Rao LV, Vankayalapati R.(2009). IL-22 produced by human NK cells inhibits growth of Mycobacterium tuberculosis by enhancing phagolysosomal fusion.J Immunol.;183(10):6639-45

Dhiman R, Indramohan M, Barnes PF, Nayak RC, Paidipally P, Rao LV, Vankayalapati R.(2009). IL-22 produced by human NK cells inhibits growth of Mycobacterium tuberculosis by enhancing phagolysosomal fusion.J Immunol.;183(10):6639-45

Dietert RR, Zelikoff JT.(2008). Early-life environment, developmental immunotoxicology, and the risk of pediatric allergic disease including asthma.Birth Defects Res B Dev Reprod Toxicol.;83(6):547-60

Duerr RH, Barmada MM, Zhang L, Davis S, Preston RA, Chensny LJ, Brown JL, Ehrlich GD,

Weeks DE, Aston CE. (1998) Linkage and association between inflammatory bowel disease and a locus on chromosome 12. Am J Hum Genet.;63(1):95-100

Duhen T, Geiger R, Jarrossay D, Lanzavecchia A, Sallusto F. (2009) Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. Nat Immunol.;10(8):857-63

Dumoutier L, Van Roost E, Ameye G, Michaux L, Renauld JC.(2000). IL-TIF/IL-22: genomic organization and mapping of the human and mouse genes.Genes Immun.;1(8):488-94

Dumoutier L, Van Roost E, Colau D, Renauld JC.(2000). Human interleukin-10-related T cell-derived inducible factor: molecular cloning and functional characterization as an hepatocyte-stimulating factor.Proc Natl Acad Sci U S A.;97(18):10144-9

Dumoutier L, Leemans C, Lejeune D, Kotenko SV, Renauld JC.(2001).Cutting edge: STAT activation by IL-19, IL-20 and mda-7 through IL-20 receptor complexes of two types.J Immunol.;167(7):3545-9

Durham SR, Varney V, Gaga M, Frew AJ, Jacobson M, Kay AB. (1991) Immunotherapy and allergic inflammation. Clin Exp Allergy.;21 Suppl 1:206-10

Eder W, Ege MJ, von Mutius E.(2006). The asthma epidemic.N Engl J Med. ;355(21):2226-35
Ege MJ, Bieli C, Frei R, van Strien RT, Riedler J, Ublagger E, Schram-Bijkerk D, Brunekreef B, van Hage M, Scheynius A, Pershagen G, Benz MR, Lauener R, von Mutius E, Braun-Fahrländer C; Parsifal Study team.(2006). Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. J Allergy Clin Immunol.;117(4):817-23

Endam LM, Bossé Y, Filali-Mouhim A, Cormier C, Boisvert P, Boulet LP, Hudson TJ, Desrosiers M. (2009) Polymorphisms in the interleukin-22 receptor alpha-1 gene are associated with severe chronic rhinosinusitis. Otolaryngol Head Neck Surg.;140(5):741-7

Essayan DM.(2001) Cyclic nucleotide phosphodiesterases.J Allergy Clin Immunol;108(5):671-80.

Ewbank PA, Murray J, Sanders K, Curran-Everett D, Dreskin S, Nelson HS.(2003). A double-blind, placebo-controlled immunotherapy dose-response study with standardized cat extract. J Allergy Clin Immunol. 2003 Jan;111(1):155-61

Eyerich S, Eyerich K, Pennino D, Carbone T, Nasorri F, Pallotta S, Cianfarani F, Odorisio T, Traidl-Hoffmann C, Behrendt H, Durham SR, Schmidt-Weber CB, Cavani A. (2009) Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. J Clin Invest.;119(12):3573-85.

Fahy JV, Fleming HE, Wong HH, Liu JT, Su JQ, Reimann J, Fick RB Jr, Boushey HA.(1997)The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects.Am J Respir Crit Care Med. ;155(6):1828-34.

Fanta CH.(2009) Asthma, N Engl J Med.;360(10):1002-14.

Feltis BN, Wignarajah D, Reid DW, Ward C, Harding R, Walters EH.(2007). Effects of inhaled fluticasone on angiogenesis and vascular endothelial growth factor in asthma.Thorax.;62(4):314-9

Figueroa-Vega N, Alfonso-Pérez M, Benedicto I, Sánchez-Madrid F, González-Amaro R, Marazuela M.(2010). Increased circulating pro-inflammatory cytokines and Th17 lymphocytes in Hashimoto's thyroiditis. J Clin Endocrinol Metab.;95(2):953-62

Fiorentino DF, Bond MW, Mosmann TR.(1989).Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones.J Exp Med. ;170(6):2081-95

Flanagan K, Modrusan Z, Cornelius J, Chavali A, Kasman I, Komuves L, Mo L, Diehl L. (2008) Intestinal epithelial cell up-regulation of LY6 molecules during colitis results in enhanced chemokine secretion. J Immunol. Mar 15;180(6):3874-81

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

Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY.(2005). A function for interleukin 2 in Foxp3-expressing regulatory T cells.Nat Immunol.;6(11):1142-51

Frew AJ, Kay AB.(1988). The pattern of human late-phase skin reactions to extracts of aeroallergens.J Allergy Clin Immunol.;81(6):1117-21

Fujimura T, Yamanashi R, Masuzawa M, Fujita Y, Katsuoka K, Nishiyama S, Mitsuyama M, Nomoto K.(1997). Conversion of the CD4+ T cell profile from T(H2)-dominant type to T(H1)-dominant type after varicella-zoster virus infection in atopic dermatitis.J Allergy Clin Immunol.;100(2):274-82

Fujita H, Nograles KE, Kikuchi T, Gonzalez J, Carucci JA, Krueger JG.(2009). Human Langerhans cells induce distinct IL-22-producing CD4+ T cells lacking IL-17 production. Proc Natl Acad Sci U S A.;106(51):21795-800

Gabrielli A, Svegliati S, Moroncini G, Luchetti M, Tonnini C, Avvedimento EV.(2007). Stimulatory autoantibodies to the PDGF receptor: a link to fibrosis in scleroderma and a pathway for novel therapeutic targets.Autoimmun Rev.;7(2):121-6

Gad HH, Dellgren C, Hamming OJ, Vends S, Paludan SR, Hartmann R.(2009). Interferon-lambda is functionally an interferon but structurally related to the interleukin-10 family.J Biol Chem.;284(31):20869-75

Gandhi R, Kumar D, Burns EJ, Nadeau M, Dake B, Laroni A, Kozoriz D, Weiner HL, Quintana FJ.(2010).Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell-like and Foxp3(+) regulatory T cells. Nat Immunol.;11(9):846-53

Gangemi S, Minciullo PL, Guarneri F, Cristani M, Arcoraci T, Spatari G, Saija A, Cannavò SP. (2009) Increased serum levels of interleukin-22 in patients affected by pityriasis rosea. J Eur Acad Dermatol Venereol.;23(7):858-9

Garat C, Kheradmand F, Albertine KH, Folkesson HG, Matthay MA. (1996) Soluble and insoluble fibronectin increases alveolar epithelial wound healing in vitro. Am J Physiol.;271(5 Pt 1):L844-53

Gauvreau GM, Watson RM, O'Byrne PM.(1999) Kinetics of allergen-induced airway eosinophilic cytokine production and airway inflammation.Am J Respir Crit Care Med.;160(2):640-7.

Gauvreau GM, Evans MY. (2007) Allergen inhalation challenge: a human model of asthma exacerbation. Contrib Microbiol.;14:21-32

Gavett SH, Chen X, Finkelman F, Wills-Karp M.(1994). Depletion of murine CD4+ T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia.Am J Respir Cell Mol Biol.;10(6):587-93

Gell PGH, Coombs RRA, (1963). Clinical Aspects of Immunology. 1st ed. Oxford, England: Blackwell

Godinez I, Haneda T, Raffatellu M, George MD, Paixão TA, Rolán HG, Santos RL, Dandekar S, Tsolis RM, Bäumler AJ.(2008). T cells help to amplify inflammatory responses induced by Salmonella enterica serotype Typhimurium in the intestinal mucosa.Infect Immun.;76(5):2008-17

Godinez I, Raffatellu M, Chu H, Paixão TA, Haneda T, Santos RL, Bevins CL, Tsolis RM, Bäumler AJ. (2009) Interleukin-23 orchestrates mucosal responses to Salmonella enterica serotype Typhimurium in the intestine. Infect Immun.;77(1):387-98

Goldstein DM, Gabriel T (2005). Pathway to the clinic: inhibition of P38 MAP kinase. A review of ten chemotypes selected for development. 5. pp. 1017–29.

Goto M, Murakawa M, Kadoshima-Yamaoka K, Tanaka Y, Nagahira K, Fukuda Y, Nishimura T.(2009). Murine NKT cells produce Th17 cytokine interleukin-22.Cell Immunol.;254(2):81-4

Gould HJ, Sutton BJ, Beavil AJ, Beavil RL, McCloskey N, Coker HA, Fear D, Smurthwaite L.(2003). The biology of IGE and the basis of allergic disease.Annu Rev Immunol.;21:579-628
Gratziou C, Carroll M, Walls A, Howarth PH, Holgate ST. (1992). Early changes in T lymphocytes recovered by bronchoalveolar lavage after local allergen challenge of asthmatic airways. Am Rev Respir Dis.;145(6):1259-64

Green RH, Brightling CE, McKenna S, et al. (2002) Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet; 360:1715-1721

Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG.(1997). A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature.;389(6652):737-42

Gu Y, Yang J, Ouyang X, Liu W, Li H, Yang J, Bromberg J, Chen SH, Mayer L, Unkeless JC, Xiong H.(2008). Interleukin 10 suppresses Th17 cytokines secreted by macrophages and T cells. Eur J Immunol.;38(7):1807-13

Guilbert TW, Morgan WJ, Zeiger RS, Mauger DT, Boehmer SJ, Szefler SJ, Bacharier LB, Lemanske RF Jr, Strunk RC, Allen DB, Bloomberg GR, Heldt G, Krawiec M, Larsen G, Liu AH, Chinchilli VM, Sorkness CA, Taussig LM, Martinez FD.(2006). Long-term inhaled corticosteroids in preschool children at high risk for asthma. N Engl J Med.;354(19):1985-97

Guo H, Topham DJ.(2010). Interleukin-22 (IL-22) production by pulmonary Natural Killer cells and the potential role of IL-22 during primary influenza virus infection.J Virol.;84(15):7750-9

Gurney AL.(2004). IL-22, a Th1 cytokine that targets the pancreas and select other peripheral tissues.Int Immunopharmacol. ;4(5):669-77

Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, Marshall RP, Bradding P, Green RH, Wardlaw AJ, Pavord ID.(2009) Mepolizumab and exacerbations of refractory eosinophilic asthma.N Engl J Med. ;360(10):973-84

Hamada H, Garcia-Hernandez Mde L, Reome JB, Misra SK, Strutt TM, McKinstry KK, Cooper AM, Swain SL, Dutton RW.(2009). Tc17, a unique subset of CD8 T cells that can protect against lethal influenza challenge. J Immunol.;182(6):3469-81

Hanson B, McGue M, Roitman-Johnson B, Segal NL, Bouchard TJ Jr, Blumenthal MN.(1991). Atopic disease and immunoglobulin E in twins reared apart and together.Am J Hum Genet.;48(5):873-9

Harper EG, Guo C, Rizzo H, Lillis JV, Kurtz SE, Skorcheva I, Purdy D, Fitch E, Iordanov M, Blauvelt A. (2009) Th17 cytokines stimulate CCL20 expression in keratinocytes in vitro and in vivo: implications for psoriasis pathogenesis. J Invest Dermatol.;129(9):2175-83

Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT.(2005). Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol.;6(11):1123-32

Hashimoto T, Akiyama K, Kobayashi N, Mori A.(2005).Comparison of IL-17 production by helper T cells among atopic and nonatopic asthmatics and control subjects. Int Arch Allergy Immunol. ;137 Suppl 1:51-4

Haudenschild CC, Schwartz SM. (1979)Endothelial regeneration. II. Restitution of endothelial continuity. Lab Invest.;41(5):407-18.

Hedrick MN, Lonsdorf AS, Shirakawa AK, Richard Lee CC, Liao F, Singh SP, Zhang HH, Grinberg A, Love PE, Hwang ST, Farber JM.(2009). CCR6 is required for IL-23-induced psoriasis-like inflammation in mice. J Clin Invest.;119(8):2317-29

Heinke S, Szücs G, Norris A, Droogmans G, Nilius B.(1995) Inhibition of volume-activated chloride currents in endothelial cells by chromones.Br J Pharmacol.115(8):1393-8.

Hennig BJ, Frodsham AJ, Hellier S, Knapp S, Yee LJ, Wright M, Zhang L, Thomas HC, Thursz M, Hill AV. (2007) Influence of IL-10RA and IL-22 polymorphisms on outcome of hepatitis C virus infection. Liver Int.;27(8):1134-43

Ho IC, Glimcher LH.(2002).Transcription: tantalizing times for T cells.Cell. ;109 Suppl:S109-20
Hoegl S, Bachmann M, Scheiermann P, Goren I, Hofstetter C, Pfeilschifter J, Zwissler B, Muhl H. (2011) Protective properties of inhaled IL-22 in a model of ventilator-induced lung injury. Am J Respir Cell Mol Biol.;44(3):369-76

Hogg JC, Eggleston PA.(1984). Is asthma an epithelial disease? Am Rev Respir Dis.;129(2):207-8

Holgate S, Casale T, Wenzel S, Bousquet J, Deniz Y, Reisner C (2005). The anti-inflammatory effects of omalizumab confirm the central role of IgE in allergic inflammation. J. Allergy Clin. Immunol. 115 (3): 459–65.

Holgate ST, Polosa R.(2008). Treatment strategies for allergy and asthma.Nat Rev Immunol.;8(3):218-30

Holgate ST, Roberts G, Arshad HS, Howarth PH, Davies DE.(2009). The role of the airway

epithelium and its interaction with environmental factors in asthma pathogenesis. Proc Am Thorac Soc.;6(8):655-9

Holgate ST.(1996). Asthma mechanisms, determinants of severity and treatment. Forward.J Allergy Clin Immunol.;98(5 Pt 2):S1-2

Holt PG, Sly PD (2007). Th2 cytokines in the asthma late-phase response. Lancet 370 (9596): 1396–8

Hosokawa Y, Hosokawa I, Ozaki K, Nakae H, Matsuo T.(2009).Cytokines differentially regulate CXCL10 production by interferon-gamma-stimulated or tumor necrosis factor-alpha-stimulated human gingival fibroblasts. J Periodontal Res.;44(2):225-31.

House of Lords - Science and Technology - Sixth Report. (2007). The Extent and Burden of Allergy in the United Kingdom. Chapter 4 (http://www.publications.parliament.uk/ pa/ ld200607/ ldselect/ ldsctech/ 166/ 16607.htm).

Hsieh CS, Macatonia SE, O'Garra A, Murphy KM.(1993). Pathogen-induced Th1 phenotype development in CD4+ alpha beta-TCR transgenic T cells is macrophage dependent. Int Immunol.;5(4):371-82

Hsing CH, Hsieh MY, Chen WY, Cheung So E, Cheng BC, Chang MS.(2006). Induction of interleukin-19 and interleukin-22 after cardiac surgery with cardiopulmonary bypass. Ann Thorac Surg.;81(6):2196-201

Hu VW, Black GE, Torres-Duarte A, Abramson FP.(2002). 3H-thymidine is a defective tool with which to measure rates of DNA synthesis. FASEB J.;16(11):1456-7.

Huang TJ, MacAry PA, Eynott P, Moussavi A, Daniel KC, Askenase PW, Kemeny DM, Chung KF.(2001).Allergen-specific Th1 cells counteract efferent Th2 cell-dependent bronchial hyperresponsiveness and eosinophilic inflammation partly via IFN-gamma. J Immunol.;166(1):207-17

Hughes T, Becknell B, Freud AG, McClory S, Briercheck E, Yu J, Mao C, Giovenzana C, Nuovo G, Wei L, Zhang X, Gavrilin MA, Wewers MD, Caligiuri MA.(2010). Interleukin-1beta selectively expands and sustains interleukin-22+ immature human natural killer cells in secondary lymphoid tissue.Immunity.;32(6):803-14

Hughes T, Becknell B, McClory S, Briercheck E, Freud AG, Zhang X, Mao H, Nuovo G, Yu J, Caligiuri MA.(2009). Stage 3 immature human natural killer cells found in secondary lymphoid tissue constitutively and selectively express the TH 17 cytokine interleukin-22.Blood.;113(17):4008-10

Hugues S.(2010).Dynamics of dendritic cell-T cell interactions: a role in T cell outcome.Semin Immunopathol.;32(3):227-38

Igawa D, Sakai M, Savan R.(2006). An unexpected discovery of two interferon gamma-like genes along with interleukin (IL)-22 and -26 from teleost: IL-22 and -26 genes have been described for the first time outside mammals.Mol Immunol. 2006 Mar;43(7):999-1009

Ikeuchi H, Kuroiwa T, Hiramatsu N, Kaneko Y, Hiromura K, Ueki K, Nojima Y. (2005). Expression of interleukin-22 in rheumatoid arthritis: potential role as a proinflammatory cytokine. Arthritis Rheum.;52(4):1037-46

Ito T, Hanabuchi S, Wang YH, Park WR, Arima K, Bover L, Qin FX, Gilliet M, Liu YJ.(2008). Two functional subsets of FOXP3+ regulatory T cells in human thymus and periphery. Immunity.;28(6):870-80

Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR.(2006). The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells.Cell.;126(6):1121-33

Jäger A, Dardalhon V, Sobel RA, Bettelli E, Kuchroo VK.(2009). Th1, Th17, and Th9 effector cells induce experimental autoimmune encephalomyelitis with different pathological phenotypes.J Immunol.;183(11):7169-77

Jain N, Covar RA, Gleason MC, Newell JD Jr, Gelfand EW, Spahn JD.(2005). Quantitative computed tomography detects peripheral airway disease in asthmatic children.Pediatr Pulmonol.;40(3):211-8

Janeway CA Jr.(1989)The priming of helper T cells.Semin Immunol. ;1(1):13-20

Johansson MW, Kelly EA, Busse WW, Jarjour NN, Mosher DF.(2008).Up-regulation and activation of eosinophil integrins in blood and airway after segmental lung antigen challenge. J Immunol.;180(11):7622-35

Johnston NW, Mandhane PJ, Dai J, Duncan JM, Greene JM, Lambert K, Sears MR.(2007). Attenuation of the September epidemic of asthma exacerbations in children: a randomized, controlled trial of montelukast added to usual therapy.Pediatrics.;120(3):e702-12

Jones BC, Logsdon NJ, Walter MR.(2008). Crystallization and preliminary X-ray diffraction analysis of human IL-22 bound to the extracellular IL-22R1 chain.Acta Crystallogr Sect F Struct Biol Cryst Commun. ;64(Pt 4):266-9

Julius P, Lommatzsch M, Kuepper M, Bratke K, Faehndrich S, Luttmann W, Virchow JC. (2008) Safety of segmental allergen challenge in human allergic asthma. J Allergy Clin Immunol.;121(3):712-7.

Juniper EF, Frith PA, Dunnett C, Cockcroft DW, Hargreave FE (1978). Reproducibility and comparison of responses to inhaled histamine and methacholine. Thorax.;33(6):705-10

Kämpe M, Janson C, Stålenheim G, Stolt I, Carlson M. (2010) Experimental and seasonal exposure to birch pollen in allergic rhinitis and allergic asthma with regard to the inflammatory response. Clin Respir J.;4(1):37-44

Kamradt T, Göggel R, Erb KJ.(2005). Induction, exacerbation and inhibition of allergic and autoimmune diseases by infection.Trends Immunol.;26(5):260-7

Kapessidou P, Poulin L, Dumoutier L, Goldman M, Renauld JC, Braun MY.(2008). Interleukin-22 deficiency accelerates the rejection of full major histocompatibility complex-disparate heart allografts. Transplant Proc.;40(5):1593-7

Kaplan MH, Schindler U, Smiley ST, Grusby MJ.(1996). Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. Immunity.;4(3):313-9

Kaplan MH, Sun YL, Hoey T, Grusby MJ.(1996). Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. Nature.;382(6587):174-7

Kay AB.(2001). Allergy and allergic diseases. N Engl J Med.;344(1):30-7 and (2):109-13

Kazani S, Israel E.(2012)Update in asthma 2011.Am J Respir Crit Care Med.;186(1):35-40.

Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, Giuliani F, Arbour N, Becher B, Prat A.(2007). Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation.Nat Med.;13(10):1173-5

Kebir H, Ifergan I, Alvarez JI, Bernard M, Poirier J, Arbour N, Duquette P, Prat A.(2009). Preferential recruitment of interferon-gamma-expressing TH17 cells in multiple sclerosis. Ann Neurol.;66(3):390-402

Kheradmand F, Folkesson HG, Shum L, Derynk R, Pytela R, Matthay MA. (1994) Transforming growth factor-alpha enhances alveolar epithelial cell repair in a new in vitro model. Am J Physiol.;267(6 Pt 1):L728-38

Kicic A, Hallstrand TS, Sutanto EN, Stevens PT, Kobor MS, Taplin C, Paré PD, Beyer RP, Stick SM, Knight DA.(2010). Decreased fibronectin production significantly contributes to dysregulated repair of asthmatic epithelium. Am J Respir Crit Care Med.;181(9):889-98

Kidney JC, Boulet LP, Hargreave FE, Deschesnes F, Swystun VA, O'Byrne PM, Choudry N, Morris MM, Jennings B, Andersson N, Andreasson A, Cockcroft DW.(1997)Evaluation of single-dose inhaled corticosteroid activity with an allergen challenge model.J Allergy Clin Immunol. ;100(1):65-70.

Killar L, MacDonald G, West J, Woods A, Bottomly K.(1987). Cloned, Ia-restricted T cells that do not produce interleukin 4(IL 4)/B cell stimulatory factor 1(BSF-1) fail to help antigen-specific B cells. J Immunol.;138(6):1674-9

Kim S, Han S, Kim MY.(2010). Effects of interleukin-15 on human CD3(-)CD117(+)CD56(-)OX40L(+) cell differentiation.Hum Immunol.;71(8):745-50

Kim SH, Matthay MA, Mostov K, Hunt CA.(2010). Simulation of lung alveolar epithelial wound healing in vitro.J R Soc Interface.;7(49):1157-70.

Kinnebrew MA, Ubeda C, Zenewicz LA, Smith N, Flavell RA, Pamer EG. (2010) Bacterial flagellin stimulates Toll-like receptor 5-dependent defense against vancomycin-resistant Enterococcus infection. J Infect Dis.;201(4):534-43

Kirby JG, Robertson DG, Hargreave FE, Dolovich J.(1986) Asthmatic responses to inhalation of anti-human IgE.Clin Allergy.;16(3):191-4.

Kisand K, Bøe Wolff AS, Podkrajsek KT, Tserel L, Link M, Kisand KV, Ersvaer E, Perheentupa J, Erichsen MM, Bratanic N, Meloni A, Cetani F, Perniola R, Ergun-Longmire B, Maclaren N, Krohn KJ, Pura M, Schalke B, Ströbel P, Leite MI, Battelino T, Husebye ES, Peterson P, Willcox N, Meager A.(2010). Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines.J Exp Med.;207(2):299-308

Kleinschek MA, Müller U, Schütze N, Sabat R, Straubinger RK, Blumenschein WM, McClanahan T, Kastelein RA, Alber G. (2010). Administration of IL-23 engages innate and adaptive immune mechanisms during fungal infection.Int Immunol.;22(2):81-90

Knight DA, Holgate ST.(2006). The airway epithelium: structural and functional properties in

health and disease. Respirology.;8(4):432-46

Koch O, Rockett K, Jallow M, Pinder M, Sisay-Joof F, Kwiatkowski D. (2005) Investigation of malaria susceptibility determinants in the IFNG/IL26/IL22 genomic region. Genes Immun.;6(4):312-8

Koch M, Witzenrath M, Reuter C, Herma M, Schütte H, Suttorp N, Collins H, Kaufmann SH.(2006).Role of local pulmonary IFN-gamma expression in murine allergic airway inflammation.Am J Respir Cell Mol Biol.;35(2):211-9

Koga C, Kabashima K, Shiraishi N, Kobayashi M, Tokura Y.(2008). Possible pathogenic role of Th17 cells for atopic dermatitis. J Invest Dermatol.;128(11):2625-30

Korn T, Bettelli E, Gao W, Awasthi A, Jäger A, Strom TB, Oukka M, Kuchroo VK.(2007). IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. Nature.;448(7152):484-7

Kotenko SV, Izotova LS, Mirochnitchenko OV, Esterova E, Dickensheets H, Donnelly RP, Pestka S.(2001).Identification of the functional interleukin-22 (IL-22) receptor complex: the IL-10R2 chain (IL-10Rbeta) is a common chain of both the IL-10 and IL-22 (IL-10-related T cell-derived inducible factor, IL-TIF) receptor complexes.J Biol Chem.;276(4):2725-32

Kraft M, Martin RJ, Lazarus SC, Fahy JV, Boushey HA, Lemanske RF Jr, Szefler SJ; Asthma Clinical Research Network.(2003). Airway tissue mast cells in persistent asthma: predictor of treatment failure when patients discontinue inhaled corticosteroids.Chest.;124(1):42-50

Kragstrup TW, Otkjaer K, Holm C, Jørgensen A, Hokland M, Iversen L, Deleuran B.(2008) The expression of IL-20 and IL-24 and their shared receptors are increased in rheumatoid arthritis and spondyloarthropathy. Cytokine. ;41(1):16-23.

Kretschmer K, Apostolou I, Hawiger D, Khazaie K, Nussenzweig MC, von Boehmer H.(2005). Inducing and expanding regulatory T cell populations by foreign antigen. Nat Immunol.;6(12):1219-27

Kreymborg K, Etzensperger R, Dumoutier L, Haak S, Rebollo A, Buch T, Heppner FL, Renauld JC, Becher B.(2007).IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. J Immunol.;179(12):8098-104

Krishan A.(1975). Rapid flow cytofluorometric analysis of mammalian cell cycle by propidium iodide staining.J Cell Biol.;66(1):188-93.

Krug N, Erpenbeck VJ, Balke K, Petschallies J, Tschernig T, Hohlfeld JM, Fabel H.(2001). Cytokine profile of bronchoalveolar lavage-derived CD4(+), CD8(+), and gammadelta T cells in people with asthma after segmental allergen challenge. Am J Respir Cell Mol Biol.;25(1):125-31

Krüger K, Mooren FC.(2007)T cell homing and exercise.Exerc Immunol Rev. 13:37-54

Kunz S, Wolk K, Witte E, Witte K, Doecke WD, Volk HD, Sterry W, Asadullah K, Sabat R. (2006) Interleukin (IL)-19, IL-20 and IL-24 are produced by and act on keratinocytes and are distinct from classical ILs. Exp Dermatol.;15(12):991-1004

Kuwano K.(2007). Epithelial cell apoptosis and lung remodeling. Cell Mol Immunol.;4(6):419-

29
Lachowicz-Scroggins ME, Boushey HA, Finkbeiner WE, Widdicombe JH. (2010). Interleukin-13-induced mucous metaplasia increases susceptibility of human airway epithelium to rhinovirus infection.Am J Respir Cell Mol Biol.;43(6):652-61
Laitinen LA, Heino M, Laitinen A, Kava T, Haahtela T.(1985).Damage of the airway epithelium and bronchial reactivity in patients with asthma. Am Rev Respir Dis.;131(4):599-606

Laitinen A, Altraja A, Kämpe M, Linden M, Virtanen I, Laitinen LA.(1997). Tenascin is increased in airway basement membrane of asthmatics and decreased by an inhaled steroid.Am J Respir Crit Care Med.;156(3 Pt 1):951-8

Laitinen T, Räsänen M, Kaprio J, Koskenvuo M, Laitinen LA.(1998).Importance of genetic factors in adolescent asthma: a population-based twin-family study.Am J Respir Crit Care Med. ;157(4 Pt 1):1073-8.

Lajoie S, Lewkowich IP, Suzuki Y, Clark JR, Sproles AA, Dienger K, Budelsky AL, Wills-Karp M.(2010).Complement-mediated regulation of the IL-17A axis is a central genetic determinant of the severity of experimental allergic asthma.Nat Immunol. 2010 Oct;11(10):928-35

Lambert, A.; Goad, B.; Pittman, D.; Clark, E.; Block, L.; Wong, T.; Erickson, J.; Hayes, L.; Sheilds, K.; Deng, B.; Spaulding, V.; Annis, B.; Zollner, R.; Wang, I.; Kobayashi, M.; Thibodeaux, D.; Leonard, J.; Jacobs, K.; Fouser, L.(2001). Novel cytokine IL-22 administrated by adenovirus vector or as recombinant purified protein induces acute-phase responses and renal tubular basophilia in female C57BL/6 mice. Toxicologic Pathology, 29 (6): 712

Lane P, Kim MY, Withers D, Gaspal F, Bekiaris V, Desanti G, Khan M, McConnell F, Anderson G.(2008). Lymphoid tissue inducer cells in adaptive CD4 T cell dependent responses.Semin Immunol.;20(3):159-63

Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ.(2005). IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med.;201(2):233-40

Larsen JM, Bonefeld CM, Poulsen SS, Geisler C, Skov L. (2009) IL-23 and T(H)17-mediated inflammation in human allergic contact dermatitis. J Allergy Clin Immunol.;123(2):486-92

Lawrence S, Beasley R, Doull I, Begishvili B, Lampe F, Holgate ST, Morton NE.(1994). Genetic analysis of atopy and asthma as quantitative traits and ordered polychotomies. Ann Hum Genet.;58(Pt 4):359-68

Le Gros G, Ben-Sasson SZ, Seder R, Finkelman FD, Paul WE.(1990). Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells.J Exp Med.;172(3):921-9

Lécart S, Morel F, Noraz N, Pène J, Garcia M, Boniface K, Lecron JC, Yssel H.(2002). IL-22, in contrast to IL-10, does not induce Ig production, due to absence of a functional IL-22 receptor on activated human B cells.Int Immunol.;14(11):1351-6

Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, Mathur AK, Cowley HC, Chung KF, Djukanovic R, Hansel TT, Holgate ST, Sterk PJ, Barnes PJ.(2000) Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response.Lancet.;356(9248):2144-8.

Leung MW, Shen S, Lafaille JJ.(2009). TCR-dependent differentiation of thymic Foxp3+ cells

is limited to small clonal sizes. J Exp Med.;206(10):2121-30

Levillayer F, Mas M, Levi-Acobas F, Brahic M, Bureau JF. (2007) Interleukin 22 is a candidate gene for Tmevp3, a locus controlling Theiler's virus-induced neurological diseases. Genetics.;176(3):1835-44

Levy ML, Thomas M, Small I, Pearce L, Pinnock H, Stephenson P. (2009). Summary of the 2008 BTS/SIGN British Guideline on the management of asthma. Prim Care Respir J.;18 Suppl 1:S1-16

Lewin B.(2008) Genes IX. Sudbury, Mass. Jones and Bartlett 2008.

Li MO, Wan YY, Flavell RA.(2007). T cell-produced transforming growth factor-beta1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation.Immunity.;26(5):579-91

Li W, Danilenko DM, Bunting S, Ganesan R, Sa S, Ferrando R, Wu TD, Kolumam GA, Ouyang W, Kirchhofer D. (2009) The serine protease marapsin is expressed in stratified squamous epithelia and is up-regulated in the hyperproliferative epidermis of psoriasis and regenerating wounds. J Biol Chem.;284(1):218-28.

Li Z, Liu B, Maminishkis A, Mahesh SP, Yeh S, Lew J, Lim WK, Sen HN, Clarke G, Buggage R, Miller SS, Nussenblatt RB.(2008). Gene expression profiling in autoimmune noninfectious uveitis disease. J Immunol.;181(7):5147-57

Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser LA.(2006). Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides.J Exp Med.;203(10):2271-9

Liang CC, Park AY, Guan JL.(2007). In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro.Nat Protoc.;2(2):329-33.

Liang SC, Long AJ, Bennett F, Whitters MJ, Karim R, Collins M, Goldman SJ, Dunussi-Joannopoulos K, Williams CM, Wright JF, Fouser LA.(2007).An IL-17F/A heterodimer protein is produced by mouse Th17 cells and induces airway neutrophil recruitment.J Immunol. ;179(11):7791-9

Lighvani AA, Frucht DM, Jankovic D, Yamane H, Aliberti J, Hissong BD, Nguyen BV, Gadina M, Sher A, Paul WE, O'Shea JJ.(2001). T-bet is rapidly induced by interferon-gamma in lymphoid and myeloid cells. Proc Natl Acad Sci U S A.;98(26):15137-42

Lilly CM, Nakamura H, Belostotsky OI, Haley KJ, Garcia-Zepeda EA, Luster AD, Israel E.(2001).Eotaxin expression after segmental allergen challenge in subjects with atopic asthma.Am J Respir Crit Care Med. ;163(7):1669-75

Lin L, Ibrahim AS, Xu X, Farber JM, Avanesian V, Baquir B, Fu Y, French SW, Edwards JE Jr, Spellberg B.(2009). Th1-Th17 cells mediate protective adaptive immunity against Staphylococcus aureus and Candida albicans infection in mice. PLoS Pathog.;5(12):e1000703

Liu Z, Yelverton RW, Kraft B, Tanner SB, Olsen NJ, Aune TM. (2005).Highly conserved gene expression profiles in humans with allergic rhinitis altered by immunotherapy.Clin Exp Allergy.;35(12):1581-90

Liu H, Huang K, Wu Y, Lin N, Li J, Tu Y. (2007) The expression of interleukin-22 and S100A7,

A8, A9 mRNA in patients with psoriasis vulgaris. J Huazhong Univ Sci Technolog Med Sci.;27(5):605-7

Lloyd CM, Robinson DS.(2007). Allergen-induced airway remodelling.Eur Respir J.;29(5):1020-32

Logsdon NJ, Jones BC, Josephson K, Cook J, Walter MR.(2002). Comparison of interleukin-22 and interleukin-10 soluble receptor complexes.J Interferon Cytokine Res.;22(11):1099-112.
Logsdon NJ, Jones BC, Allman JC, Izotova L, Schwartz B, Pestka S, Walter MR.(2004). The IL-10R2 binding hot spot on IL-22 is located on the N-terminal helix and is dependent on N-linked glycosylation. J Mol Biol.;342(2):503-14

Lötvall J, Akdis CA, Bacharier LB, Bjermer L, Casale TB, Custovic A, Lemanske RF Jr, Wardlaw AJ, Wenzel SE, Greenberger PA.(2011)Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome.J Allergy Clin Immunol.;127(2):355-60.

Lowes MA, Kikuchi T, Fuentes-Duculan J, Cardinale I, Zaba LC, Haider AS, Bowman EP, Krueger JG. (2008) Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. J Invest Dermatol.;128(5):1207-11

Luci C, Reynders A, Ivanov II, Cognet C, Chiche L, Chasson L, Hardwigsen J, Anguiano E, Banchereau J, Chaussabel D, Dalod M, Littman DR, Vivier E, Tomasello E.(2009). Influence of the transcription factor RORgammat on the development of NKp46+ cell populations in gut and skin. Nat Immunol.;10(1):75-82

Lundgren R, Söderberg M, Hörstedt P, Stenling R.(1988). Morphological studies of bronchial mucosal biopsies from asthmatics before and after ten years of treatment with inhaled steroids.Eur Respir J.;1(10):883-9

Ma HL, Napierata L, Stedman N, Benoit S, Collins M, Nickerson-Nutter C, Young DA.(2010). Tumor necrosis factor alpha blockade exacerbates murine psoriasis-like disease by enhancing Th17 function and decreasing expansion of Treg cells. Arthritis Rheum.;62(2):430-40.

Manel N, Unutmaz D, Littman DR.(2008). The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat.Nat Immunol.;9(6):641-9

Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT.(2006). Transforming growth factor-beta induces development of the T(H)17 lineage. Nature.;441(7090):231-4

Martin B, Hirota K, Cua DJ, Stockinger B, Veldhoen M.(2009). Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. Immunity.;31(2):321-30

Martin-Orozco N, Chung Y, Chang SH, Wang YH, Dong C.(2009). Th17 cells promote pancreatic inflammation but only induce diabetes efficiently in lymphopenic hosts after conversion into Th1 cells.Eur J Immunol.;39(1):216-24

Matsumoto K, Narita S, Rerecich T, Snider DP, O'Byrne PM.(2004).Different profile of interleukin-10 production in circulating T cells from atopic asthmatics compared with healthy subjects.Can Respir J.;11(1):33-8

Matthews K, Wilkinson KA, Kalsdorf B, Roberts T, Diacon A, Walzl G, Wolske J, Ntsekhe M, Syed F, Russell J, Mayosi BM, Dawson R, Dheda K, Wilkinson RJ, Hanekom WA, Scriba TJ.(2011). Predominance of interleukin-22 over interleukin-17 at the site of disease in human tuberculosis. Tuberculosis (Edinb).;91(6):587-93

Maxmen A (2011).Asthma: Breathing new life into research. Nature 479, S20–S21

Maziak W. (2005) The asthma epidemic and our artificial habitats.BMC Pulm Med. ;5:5

McGeachy MJ, Cua DJ.(2007). The link between IL-23 and Th17 cell-mediated immune pathologies.Semin Immunol.;19(6):372-6

McKay L.I., Cidlowski J.A. (1999) Molecular control of immune/inflammatory responses: Interactions between NF-?B and steroid receptor-signaling pathways. Endocrinol. Rev.;20:435–459.

Mebius RE, Rennert P, Weissman IL.(1997). Developing lymph nodes collect CD4+CD3- LTbeta+ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells. Immunity.;7(4):493-504

Meyer KC. (2007) Bronchoalveolar lavage as a diagnostic tool. Semin Respir Crit Care Med.;28(5):546-60

Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA.(2010). An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. J Immunol.;185(6):3190-8

Missé D, Yssel H, Trabattoni D, Oblet C, Lo Caputo S, Mazzotta F, Pène J, Gonzalez JP, Clerici M, Veas F. (2007) IL-22 participates in an innate anti-HIV-1 host-resistance network through acute-phase protein induction. J Immunol.;178(1):407-15

Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, Depner M, von Berg A, Bufe A, Rietschel E, Heinzmann A, Simma B, Frischer T, Willis-Owen SA, Wong KC, Illig T, Vogelberg C, Weiland SK, von Mutius E, Abecasis GR, Farrall M, Gut IG, Lathrop GM, Cookson WO.(2007). Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma.Nature.;448(7152):470-3

Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, von Mutius E, Farrall M, Lathrop M, Cookson WO; GABRIEL Consortium.(2010). A large-scale, consortium-based genomewide association study of asthma.N Engl J Med.;363(13):1211-21

Molet S, Hamid Q, Davoine F, Nutku E, Taha R, Pagé N, Olivenstein R, Elias J, Chakir J.(2001).IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. J Allergy Clin Immunol. ;108(3):430-8

Montefort S, Roberts JA, Beasley R, Holgate ST, Roche WR.(1992). The site of disruption of the bronchial epithelium in asthmatic and non-asthmatic subjects. Thorax.;47(7):499-503

Montuschi P, Peters-Golden ML.(2010) Leukotriene modifiers for asthma treatment.Clin Exp Allergy.;40(12):1732-41

Moorman JE, Rudd RA, Johnson CA, King M, Minor P, Bailey C, Scalia MR, Akinbami LJ; Centers for Disease Control and Prevention (CDC).(2007). National surveillance for asthma--

United States, 1980-2004.MMWR Surveill Summ.;56(8):1-54

Morcillo EJ, Cortijo J.(2006). Mucus and MUC in asthma.Curr Opin Pulm Med.;12(1):1-6

Morgan AJ, Symon FA, Berry MA, Pavord ID, Corrigan CJ, Wardlaw AJ.(2005).IL-4-expressing bronchoalveolar T cells from asthmatic and healthy subjects preferentially express CCR 3 and CCR 4. J Allergy Clin Immunol. ;116(3):594-600

Morishima Y, Nomura A, Uchida Y, Noguchi Y, Sakamoto T, Ishii Y, Goto Y, Masuyama K, Zhang MJ, Hirano K, Mochizuki M, Ohtsuka M, Sekizawa K.(2001). Triggering the induction of myofibroblast and fibrogenesis by airway epithelial shedding. Am J Respir Cell Mol Biol.;24(1):1-11

Mukherjee S, Schaller MA, Neupane R, Kunkel SL, Lukacs NW.(2009). Regulation of T cell activation by Notch ligand, DLL4, promotes IL-17 production and Rorc activation.J Immunol. 182(12):7381-8

Mullings RE, Wilson SJ, Puddicombe SM, Lordan JL, Bucchieri F, Djukanović R, Howarth PH, Harper S, Holgate ST, Davies DE.(2001). Signal transducer and activator of transcription 6 (STAT-6) expression and function in asthmatic bronchial epithelium.J Allergy Clin Immunol.;108(5):832-8

Muñoz M, Heimesaat MM, Danker K, Struck D, Lohmann U, Plickert R, Bereswill S, Fischer A, Dunay IR, Wolk K, Loddenkemper C, Krell HW, Libert C, Lund LR, Frey O, Hölscher C, Iwakura Y, Ghilardi N, Ouyang W, Kamradt T, Sabat R, Liesenfeld O.(2009). Interleukin (IL)-23 mediates Toxoplasma gondii-induced immunopathology in the gut via matrixmetalloproteinase-2 and IL-22 but independent of IL-17. J Exp Med.;206(13):3047-59

Murray CS, Woodcock A, Langley SJ, Morris J, Custovic A; IFWIN study team.(2006). Secondary prevention of asthma by the use of Inhaled Fluticasone propionate in Wheezy INfants (IFWIN): double-blind, randomised, controlled study.Lancet.;368(9537):754-62

Nagalakshmi ML, Murphy E, McClanahan T, de Waal Malefyt R. (2004) Expression patterns of IL-10 ligand and receptor gene families provide leads for biological characterization. Int Immunopharmacol.;4(5):577-92

Nagem RA, Colau D, Dumoutier L, Renauld JC, Ogata C, Polikarpov I.(2002). Crystal structure of recombinant human interleukin-22.Structure.;10(8):1051-62

Naish S, Boenisch T, Farmilo AJ, Stead RH. (1989). Handbook of immunological staining methods. Dako Cooperation

Nakagome K, Imamura M, Kawahata K, Harada H, Okunishi K, Matsumoto T, Sasaki O, Tanaka R, Kano MR, Chang H, Hanawa H, Miyazaki J, Yamamoto K, Dohi M.(2011). High expression of IL-22 suppresses antigen-induced immune responses and eosinophilic airway inflammation via an IL-10-associated mechanism. J Immunol.;187(10):5077-89

Nakamura M, Tokura Y. (2010) A novel missense mutation in tumour necrosis factor receptor superfamily 1A (TNFRSF1A) gene found in tumour necrosis factor receptor-associated periodic syndrome (TRAPS) with high serum interleukin (IL)-22. Eur J Dermatol.;20(4):508-9.

Nelson HS, Bernstein IL, Fink J, Edwards TB, Spector SL, Storms WW, Tashkin DP.(1998). Oral glucocorticosteroid-sparing effect of budesonide administered by Turbuhaler: a double-blind, placebo-controlled study in adults with moderate-to-severe chronic asthma. Pulmicort

Turbuhaler Study Group.Chest.;113(5):1264-71

Ness-Schwickerath KJ, Jin C, Morita CT.(2010). Cytokine requirements for the differentiation and expansion of IL-17A- and IL-22-producing human Vgamma2Vdelta2 T cells. J Immunol.;184(12):7268-80

Neufert C, Pickert G, Zheng Y, Wittkopf N, Warntjen M, Nikolaev A, Ouyang W, Neurath MF, Becker C. (2010) Activation of epithelial STAT3 regulates intestinal homeostasis. Cell Cycle.;9(4):652-5

Newcomb DC, Boswell MG, Huckabee MM, Goleniewska K, Dulek DE, Reiss S, Lukacs NW, Kolls JK, Peebles RS Jr.(2012).IL-13 regulates Th17 secretion of IL-17A in an IL-10-dependent manner. J Immunol.;188(3):1027-35

Nishihara M, Ogura H, Ueda N, Tsuruoka M, Kitabayashi C, Tsuji F, Aono H, Ishihara K, Huseby E, Betz UA, Murakami M, Hirano T.(2007). IL-6-gp130-STAT3 in T cells directs the development of IL-17+ Th with a minimum effect on that of Treg in the steady state. Int Immunol.;19(6):695-702

Nistala K, Moncrieffe H, Newton KR, Varsani H, Hunter P, Wedderburn LR. (2008) 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 Rheum.;58(3):875-87

Noal RB, Menezes AM, Macedo SE, Dumith SC.(2011). Childhood body mass index and risk of asthma in adolescence: a systematic review. Obes Rev.;12(2):93-104

Noben-Trauth N, Hu-Li J, Paul WE.(2002). IL-4 secreted from individual naive CD4+ T cells acts in an autocrine manner to induce Th2 differentiation.Eur J Immunol.;32(5):1428-33

Nograles KE, Zaba LC, Guttman-Yassky E, Fuentes-Duculan J, Suárez-Fariñas M, Cardinale I, Khatcherian A, Gonzalez J, Pierson KC, White TR, Pensabene C, Coats I, Novitskaya I, Lowes MA, Krueger JG. (2008). Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. Br J Dermatol.;159(5):1092-102

Nograles KE, Zaba LC, Shemer A, Fuentes-Duculan J, Cardinale I, Kikuchi T, Ramon M, Bergman R, Krueger JG, Guttman-Yassky E. (2009) IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. J Allergy Clin Immunol.;123(6):1244-52.e2

Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, Schluns K, Tian Q, Watowich SS, Jetten AM, Dong C.(2007). Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature.;448(7152):480-3

Nuttall SL, Langford NJ, Kendall MJ.(2000). Frequent paracetamol use linked to asthma. Lancet.;355(9215):1648-9

Ober C, Cox NJ, Abney M, Di Rienzo A, Lander ES, Changyaleket B, Gidley H, Kurtz B, Lee J, Nance M, Pettersson A, Prescott J, Richardson A, Schlenker E, Summerhill E, Willadsen S, Parry R. (1998) Genome-wide search for asthma susceptibility loci in a founder population. The Collaborative Study on the Genetics of Asthma. Hum Mol Genet.;7(9):1393-8

O'Byrne PM, Dolovich J, Hargreave FE.(1987)Late asthmatic responses.Am Rev Respir Dis. 1987 Sep;136(3):740-51

O'Byrne PM, Gavreau GM, Brannan JD. (2009) Provoked models of asthma: what have we learnt? Clin Exp Allergy; 39:181-92

O'Byrne PM, Pedersen S, Lamm CJ, Tan WC, Busse WW; START Investigators Group.(2009b). Severe exacerbations and decline in lung function in asthma. Am J Respir Crit Care Med.;179(1):19-24

O'Garra A, McEvoy LM, Zlotnik A.(1998).T-cell subsets: chemokine receptors guide the way.Curr Biol. 1;8(18):R646-9

Olivieri D, Chetta A, Del Donno M, Bertorelli G, Casalini A, Pesci A, Testi R, Foresi A.(1997). Effect of short-term treatment with low-dose inhaled fluticasone propionate on airway inflammation and remodeling in mild asthma: a placebo-controlled study.Am J Respir Crit Care Med.;155(6):1864-71

Olsavsky KM, Page JL, Johnson MC, Zarbl H, Strom SC, Omiecinski CJ. (2007) Gene expression profiling and differentiation assessment in primary human hepatocyte cultures, established hepatoma cell lines, and human liver tissues. Toxicol Appl Pharmacol.;222(1):42-56.

Openshaw PJ, Hewitt C.(2000)Protective and harmful effects of viral infections in childhood on wheezing disorders and asthma.Am J Respir Crit Care Med.;162(2 Pt 2):S40-3.

Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, Vega F, Yu N, Wang J, Singh K, Zonin F, Vaisberg E, Churakova T, Liu M, Gorman D, Wagner J, Zurawski S, Liu Y, Abrams JS, Moore KW, Rennick D, de Waal-Malefyt R, Hannum C, Bazan JF, Kastelein RA.(2000). Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. Immunity.;13(5):715-25

Oseroff C, Sidney J, Kotturi MF, Kolla R, Alam R, Broide DH, Wasserman SI, Weiskopf D, McKinney DM, Chung JL, Petersen A, Grey H, Peters B, Sette A.(2010). Molecular determinants of T cell epitope recognition to the common Timothy grass allergen. J Immunol.;185(2):943-55

Osorio F, LeibundGut-Landmann S, Lochner M, Lahl K, Sparwasser T, Eberl G, Reis e Sousa C.(2008). DC activated via dectin-1 convert Treg into IL-17 producers.Eur J Immunol.;38(12):3274-81

Ostrom NK, Decotiis BA, Lincourt WR, Edwards LD, Hanson KM, Carranza Rosenzweig JR, Crim C.(2005). Comparative efficacy and safety of low-dose fluticasone propionate and montelukast in children with persistent asthma. J Pediatr.;147(2):213-20

Paganin F, Séneterre E, Chanez P, Daurés JP, Bruel JM, Michel FB, Bousquet J.(1996). Computed tomography of the lungs in asthma: influence of disease severity and etiology. Am J Respir Crit Care Med.;153(1):110-4

Pagliaccetti NE, Chu EN, Bolen CR, Kleinstein SH, Robek MD.(2010). Lambda and alpha interferons inhibit hepatitis B virus replication through a common molecular mechanism but with different in vivo activities. Virology.;401(2):197-206

Pan C, Kumar C, Bohl S, Klingmueller U, Mann M. (2009) Comparative proteomic phenotyping of cell lines and primary cells to assess preservation of cell type-specific functions. Mol Cell Proteomics.;8(3):443-50

Pan HF, Zhao XF, Yuan H, Zhang WH, Li XP, Wang GH, Wu GC, Tang XW, Li WX, Li LH, Feng JB, Hu CS, Ye DQ. (2009) Decreased serum IL-22 levels in patients with systemic lupus erythematosus. Clin Chim Acta.;401(1-2):179-80

Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, Wang Y, Hood L, Zhu Z, Tian Q, Dong C.(2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol.;6(11):1133-41

Patel MM, Miller RL.(2009). Air pollution and childhood asthma: recent advances and future directions.Curr Opin Pediatr.;21(2):235-42

Pauwels RA, Pedersen S, Busse WW, Tan WC, Chen YZ, Ohlsson SV, Ullman A, Lamm CJ, O'Byrne PM; START Investigators Group.(2003). Early intervention with budesonide in mild persistent asthma: a randomised, double-blind trial.Lancet.;361(9363):1071-6

Pavord ID, Tattersfield AE.(1995)Bronchoprotective role for endogenous prostaglandin E2.Lancet.;345(8947):436-8.

Payne DN, Qiu Y, Zhu J, Peachey L, Scallan M, Bush A, Jeffery PK.(2004). Airway inflammation in children with difficult asthma: relationships with airflow limitation and persistent symptoms.Thorax.;59(10):862-9

Pearce N, Aït-Khaled N, Beasley R, Mallol J, Keil U, Mitchell E, Robertson C; and the ISAAC Phase Three Study Group.(2007). Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC).Thorax.;62(9):758-66

Pease JE.(2006). Asthma, allergy and chemokines.Curr Drug Targets.;7(1):3-12

Pelaia G, Vatrella A, Maselli R. (2012) The potential of biologics for the treatment of asthma. Nat Rev Drug Discov.;11(12):958-72

Pène J, Chevalier S, Preisser L, Vénéreau E, Guilleux MH, Ghannam S, Molès JP, Danger Y, Ravon E, Lesaux S, Yssel H, Gascan H. (2008) Chronically inflamed human tissues are infiltrated by highly differentiated Th17 lymphocytes. J Immunol. ;180(11):7423-30

Pergola C, Werz O.(2010) 5-Lipoxygenase inhibitors: a review of recent developments and patents.Expert Opin Ther Pat.;20(3):355-75.

Pickert G, Neufert C, Leppkes M, Zheng Y, Wittkopf N, Warntjen M, Lehr HA, Hirth S, Weigmann B, Wirtz S, Ouyang W, Neurath MF, Becker C.(2009). STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. J Exp Med.;206(7):1465-72.

Pilette C, Francis JN, Till SJ, Durham SR.(2004).CCR4 ligands are up-regulated in the airways of atopic asthmatics after segmental allergen challenge.Eur Respir J. ;23(6):876-84
Piriyapongsa J, Polavarapu N, Borodovsky M, McDonald J. (2007) Exonization of the LTR transposable elements in human genome.BMC Genomics.;8:291

Placek K, Coffre M, Maiella S, Bianchi E, Rogge L.(2009). Genetic and epigenetic networks controlling T helper 1 cell differentiation.Immunology.;127(2):155-62

Platts-Mills TA, Erwin E, Heymann P, Woodfolk J.(2005). Is the hygiene hypothesis still a viable explanation for the increased prevalence of asthma?Allergy.;60 Suppl 79:25-31

Poon AH, Hamid Q.(2012) Asthma endotypes: the right direction towards personalized medicine for asthma.Expert Rev Clin Immunol.;8(7):595-6

Puel A, Döffinger R, Natividad A, Chrabieh M, Barcenas-Morales G, Picard C, Cobat A, Ouachée-Chardin M, Toulon A, Bustamante J, Al-Muhsen S, Al-Owain M, Arkwright PD, Costigan C, McConnell V, Cant AJ, Abinun M, Polak M, Bougnères PF, Kumararatne D, Marodi L, Nahum A, Roifman C, Blanche S, Fischer A, Bodemer C, Abel L, Lilic D, Casanova JL.(2010). Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I.J Exp Med.;207(2):291-7

Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC.(1993). Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society.Eur Respir J Suppl.;16:5-40

Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, Bettelli E, Caccamo M, Oukka M, Weiner HL.(2008). Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. Nature.;453(7191):65-71

Radaeva S, Sun R, Pan HN, Hong F, Gao B. (2004) Interleukin 22 (IL-22) plays a protective role in T cell-mediated murine hepatitis: IL-22 is a survival factor for hepatocytes via STAT3 activation. Hepatology.;39(5):1332-42

Raemdonck K, de Alba J, Birrell MA, Grace M, Maher SA, Irvin CG, Fozard JR, O'Byrne PM, Belvisi MG. (2012) A role for sensory nerves in the late asthmatic response. Thorax;67(1):19-25.

Ramanathan M Jr, Spannhake EW, Lane AP.(2007). Chronic rhinosinusitis with nasal polyps is associated with decreased expression of mucosal interleukin 22 receptor. Laryngoscope.;117(10):1839-43

Randolph DA, Stephens R, Carruthers CJ, Chaplin DD.(1999). Cooperation between Th1 and Th2 cells in a murine model of eosinophilic airway inflammation.J Clin Invest.;104(8):1021-9

Rätsep R, Kingo K, Karelson M, Reimann E, Raud K, Silm H, Vasar E, Kõks S. (2008) Gene expression study of IL10 family genes in vitiligo skin biopsies, peripheral blood mononuclear cells and sera. Br J Dermatol. 2008;159(6):1275-81

Ren X, Hu B, Colletti LM. (2010) IL-22 is involved in liver regeneration after hepatectomy. Am J Physiol Gastrointest Liver Physiol.;298(1):G74-80.

Rezaei N. (2006) TNF-receptor-associated periodic syndrome (TRAPS): an autosomal dominant multisystem disorder. Clin Rheumatol.;25(6):773-7

Rhen T, Cidlowski JA.(2005) Antiinflammatory action of glucocorticoids--new mechanisms for old drugs.N Engl J Med. ;353(16):1711-23.

Ricciardi L, Minciullo PL, Saitta S, Trombetta D, Saija A, Gangemi S. (2009) Increased serum levels of IL-22 in patients with nickel contact dermatitis. Contact Dermatitis.;60(1):57-8

Robertson DG, Kerigan AT, Hargreave FE, Chalmers R, Dolovich J.(1974)Late asthmatic responses induced by ragweed pollen allergen. J Allergy Clin Immunol.;54(4):244-54

Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR (1993). 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.;92(2):313-24

Robinson DS, Larché M, Durham SR.(2004).Tregs and allergic disease.J Clin Invest. ;114(10):1389-97

Robinson DS, Tsicopoulos A, Meng Q, Durham S, Kay AB, Hamid Q.(1996).Increased interleukin-10 messenger RNA expression in atopic allergy and asthma.Am J Respir Cell Mol Biol.;14(2):113-7

Robinson DS.(2009). Regulatory T cells and asthma. Clin Exp Allergy.;39(9):1314-23

Romagnani S.(1991). Human TH1 and TH2 subsets: doubt no more.Immunol Today.;12(8):256-7

Romagnani S.(2002). Cytokines and chemoattractants in allergic inflammation.Mol Immunol.;38(12-13):881-5

Rosenkranz MA, Busse WW, Sheridan JF, Crisafi GM, Davidson RJ.(2012) Are there neurophenotypes for asthma? Functional brain imaging of the interaction between emotion and inflammation in asthma. PLoS One.;7(8):e40921

Rothenberg EV, Taghon T.(2005)Molecular genetics of T cell development.Annu Rev Immunol.;23:601-49

Rudloff I, Bachmann M, Pfeilschifter J, Mühl H.(2012). Mechanisms of rapid induction of interleukin-22 in activated T cells and its modulation by cyclosporin a. J Biol Chem.;287(7):4531-43

Sa SM, Valdez PA, Wu J, Jung K, Zhong F, Hall L, Kasman I, Winer J, Modrusan Z, Danilenko DM, Ouyang W.(2007). The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. J Immunol.;178(4):2229-40.

Sackesen C, Ercan H, Dizdar E, Soyer O, Gumus P, Tosun BN, Büyüktuncer Z, Karabulut E, Besler T, Kalayci O.(2008). A comprehensive evaluation of the enzymatic and nonenzymatic antioxidant systems in childhood asthma. J Allergy Clin Immunol.;122(1):78-85

Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M.(1995). Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases.J Immunol.;155(3):1151-64

Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A, Bluestone JA.(2000). B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. Immunity.;12(4):431-40

Sampath D, Castro M, Look DC, Holtzman MJ.(1999). Constitutive activation of an epithelial signal transducer and activator of transcription (STAT) pathway in asthma.J Clin Invest.;103(9):1353-61

Sandberg ML, Sutton SE, Pletcher MT, Wiltshire T, Tarantino LM, Hogenesch JB, Cooke

MP.(2005) c-Myb and p300 regulate hematopoietic stem cell proliferation and differentiation. Dev Cell.;8(2):153-66.

Sandberg S, Paton JY, Ahola S, McCann DC, McGuinness D, Hillary CR, Oja H.(2000). The role of acute and chronic stress in asthma attacks in children.Lancet.;356(9234):982-7

Sanos SL, Bui VL, Mortha A, Oberle K, Heners C, Johner C, Diefenbach A.(2009). RORgammat and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46+ cells.Nat Immunol.;10(1):83-91

Sato TN, Tozawa Y, Deutsch U, Wolburg-Buchholz K, Fujiwara Y, Gendron-Maguire M, Gridley T, Wolburg H, Risau W, Qin Y.(1995). Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation.Nature.;376(6535):70-4.

Satoh-Takayama N, Vosshenrich CA, Lesjean-Pottier S, Sawa S, Lochner M, Rattis F, Mention JJ, Thiam K, Cerf-Bensussan N, Mandelboim O, Eberl G, Di Santo JP.(2008). Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. Immunity.;29(6):958-70

Satoh-Takayama N, Dumoutier L, Lesjean-Pottier S, Ribeiro VS, Mandelboim O, Renauld JC, Vosshenrich CA, Di Santo JP. (2009) The natural cytotoxicity receptor NKp46 is dispensable for IL-22-mediated innate intestinal immune defense against Citrobacter rodentium. J Immunol. Nov 15;183(10):6579-87

Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP.(1996). Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12.Nat Genet.;14(2):199-202

Schmechel S, Konrad A, Diegelmann J, Glas J, Wetzke M, Paschos E, Lohse P, Göke B, Brand S. (2008) Linking genetic susceptibility to Crohn's disease with Th17 cell function: IL-22 serum levels are increased in Crohn's disease and correlate with disease activity and IL23R genotype status. Inflamm Bowel Dis.;14(2):204-12

Schmidt-Weber CB, Akdis M, Akdis CA.(2007). TH17 cells in the big picture of immunology. J Allergy Clin Immunol.;120(2):247-54

Schnyder B, Lima C, Schnyder-Candrian S. (2010) Interleukin-22 is a negative regulator of the allergic response. Cytokine.;50(2):220-7

Schultz EN, Devadason SG, Khoo SK, Zhang G, Bizzintino JA, Martin AC, Goldblatt J, Laing IA, Le Souëf PN, Hayden CM.(2010). The role of GSTP1 polymorphisms and tobacco smoke exposure in children with acute asthma.J Asthma.;47(9):1049-56.

Schulz SM, Köhler G, Schütze N, Knauer J, Straubinger RK, Chackerian AA, Witte E, Wolk K, Sabat R, Iwakura Y, Holscher C, Müller U, Kastelein RA, Alber G. (2008) Protective immunity to systemic infection with attenuated Salmonella enterica serovar enteritidis in the absence of IL-12 is associated with IL-23-dependent IL-22, but not IL-17. J Immunol. ;181(11):7891-901

Scriba TJ, Kalsdorf B, Abrahams DA, Isaacs F, Hofmeister J, Black G, Hassan HY, Wilkinson RJ, Walzl G, Gelderbloem SJ, Mahomed H, Hussey GD, Hanekom WA.(2008). Distinct, specific IL-17- and IL-22-producing CD4+ T cell subsets contribute to the human anti-mycobacterial immune response.J Immunol.;180(3):1962-70

Sekikawa A, Fukui H, Suzuki K, Karibe T, Fujii S, Ichikawa K, Tomita S, Imura J, Shiratori K, Chiba T, Fujimori T. (2010) Involvement of the IL-22/REG Ialpha axis in ulcerative colitis. Lab Invest.;90(3):496-505

Sellge G, Magalhaes JG, Konradt C, Fritz JH, Salgado-Pabon W, Eberl G, Bandeira A, Di Santo JP, Sansonetti PJ, Phalipon A.(2010). Th17 cells are the dominant T cell subtype primed by Shigella flexneri mediating protective immunity. J Immunol.;184(4):2076-85

Shamji MH, Durham SR.(2011).Mechanisms of immunotherapy to aeroallergens.Clin Exp Allergy.;41(9):1235-46

Shevach EM.(2009). Mechanisms of foxp3+ T regulatory cell-mediated suppression.Immunity.;30(5):636-45

Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, Chu C, Quelle FW, Nosaka T, Vignali DA, Doherty PC, Grosveld G, Paul WE, Ihle JN.(1996). Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature.;380(6575):630-3

Siegel MD, Zhang DH, Ray P, Ray A.(1995). Activation of the interleukin-5 promoter by cAMP in murine EL-4 cells requires the GATA-3 and CLE0 elements. J Biol Chem. 1995 Oct 13;270(41):24548-55

Siegemund S, Schütze N, Schulz S, Wolk K, Nasilowska K, Straubinger RK, Sabat R, Alber G.(2009). Differential IL-23 requirement for IL-22 and IL-17A production during innate immunity against Salmonella enterica serovar Enteritidis. Int Immunol.;21(5):555-65

Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F, Kjellman B.(2005). Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. Am J Respir Crit Care Med.;171(2):137-41

Simons FE.(1997). A comparison of beclomethasone, salmeterol, and placebo in children with asthma. Canadian Beclomethasone Dipropionate-Salmeterol Xinafoate Study Group.N Engl J Med.;337(23):1659-65

Skadhauge LR, Christensen K, Kyvik KO, Sigsgaard T.(1999). Genetic and environmental influence on asthma: a population-based study of 11,688 Danish twin pairs. Eur Respir J.;13(1):8-14

Sonnenberg GF, Nair MG, Kirn TJ, Zaph C, Fouser LA, Artis D. (2010) Pathological versus protective functions of IL-22 in airway inflammation are regulated by IL-17A. J Exp Med.;207(6):1293-305. Epub 2010 May 24.

Souwer Y, Szegedi K, Kapsenberg ML, de Jong EC. 2010 IL-17 and IL-22 in atopic allergic disease. Curr Opin Immunol.;22(6):821-6

Spina D (2008) PDE4 inhibitors: current status; British Journal of Pharmacology 155 (3): 308–315

Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ.(1990). Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. N Engl J Med.;323(8):502-7

Strachan DP (1989). Hay fever, hygiene, and household size.BMJ.;299(6710):1259-60

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

Sugimoto K, Ogawa A, Mizoguchi E, Shimomura Y, Andoh A, Bhan AK, Blumberg RS, Xavier RJ, Mizoguchi A. (2008) IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. J Clin Invest.;118(2):534-44

Sutton CE, Lalor SJ, Sweeney CM, Brereton CF, Lavelle EC, Mills KH.(2009). Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. Immunity.;31(2):331-41

Swain SL, Weinberg AD, English M, Huston G.(1990). IL-4 directs the development of Th2-like helper effectors.J Immunol.;145(11):3796-806

Swain SL, Bradley LM.(1992).Helper T cell memory: more questions than answers.Semin Immunol. 1992 Feb;4(1):59-68

Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH.(2000). A novel transcription factor, T-bet, directs Th1 lineage commitment.Cell.;100(6):655-69

Tai X, Cowan M, Feigenbaum L, Singer A.(2005). CD28 costimulation of developing thymocytes induces Foxp3 expression and regulatory T cell differentiation independently of interleukin 2. Nat Immunol.;6(2):152-62

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

Takatori H, Kanno Y, Watford WT, Tato CM, Weiss G, Ivanov II, Littman DR, O'Shea JJ.(2009). Lymphoid tissue inducer-like cells are an innate source of IL-17 and IL-22. J Exp Med.;206(1):35-41

Takeda K, Kamanaka M, Tanaka T, Kishimoto T, Akira S.(1996). Impaired IL-13-mediated functions of macrophages in STAT6-deficient mice. J Immunol.;157(8):3220-2

Takhar P, Smurthwaite L, Coker HA, Fear DJ, Banfield GK, Carr VA, Durham SR, Gould HJ. (2005). Allergen drives class switching to IgE in the nasal mucosa in allergic rhinitis. J Immunol. ;174(8):5024-32

Tamura G, Satoh K, Chao CL, Inoue H, Takishima T. (1991) Do diagnostic procedures other than inhalation challenge predict immediate bronchial responses to inhaled allergen? Clin Exp Allergy.;21(4):497-502

Tan FK.(2006). Autoantibodies against PDGF receptor in scleroderma. N Engl J Med.;354(25):2709-11.

Taylor DR.(2006) Pharmacogenetics of beta2-agonist drugs in asthma. Clin Rev Allergy Immunol.;31(2-3):247-58

Teran LM, Carroll MP, Shute JK, Holgate ST. (1999). Interleukin 5 release into asthmatic airways 4 and 24 hours after endobronchial allergen challenge: its relationship with eosinophil recruitment. Cytokine.;11(7):518-22

Thessen Hedreul M, Gillett A, Olsson T, Jagodic M, Harris RA.(2009). Characterization of

Multiple Sclerosis candidate gene expression kinetics in rat experimental autoimmune encephalomyelitis. J Neuroimmunol.;210(1-2):30-9

Thierfelder WE, van Deursen JM, Yamamoto K, Tripp RA, Sarawar SR, Carson RT, Sangster MY, Vignali DA, Doherty PC, Grosveld GC, Ihle JN.(1996). Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. Nature.;382(6587):171-4

Thompson CL, Plummer SJ, Tucker TC, Casey G, Li L. (2010) Interleukin-22 genetic polymorphisms and risk of colon cancer. Cancer Causes Control.;21(8):1165-70

Thompson JS, Chu Y, Glass JF, Brown SA. (2010) Absence of IL-23p19 in donor allogeneic cells reduces mortality from acute GVHD. Bone Marrow Transplant.;45(4):712-22

Thunberg S, Gafvelin G, Nord M, Grönneberg R, Grunewald J, Eklund A, van Hage M.(2010).Allergen provocation increases TH2-cytokines and FOXP3 expression in the asthmatic lung.Allergy.;65(3):311-8

Till S, Durham S, Dickason R, Huston D, Bungre J, Walker S, Robinson D, Kay AB, Corrigan C.(1997). IL-13 production by allergen-stimulated T cells is increased in allergic disease and associated with IL-5 but not IFN-gamma expression.Immunology.;91(1):53-7

Till SJ, Durham SR, Rajakulasingam K, Humbert M, Huston D, Dickason R, Kay AB, Corrigan CJ.(1998). Allergen-induced proliferation and interleukin-5 production by bronchoalveolar lavage and blood T cells after segmental allergen challenge. Am J Respir Crit Care Med.;158(2):404-11.

Tilley SL, Hartney JM, Erikson CJ, Jania C, Nguyen M, Stock J, McNeisch J, Valancius C, Panettieri RA Jr, Penn RB, Koller BH.(2003) Receptors and pathways mediating the effects of prostaglandin E2 on airway tone.Am J Physiol Lung Cell Mol Physiol.;284(4):L599-606

Tzivian L.(2011).Outdoor air pollution and asthma in children.J Asthma.;48(5):470-81.

Todaro GJ, Lazar GK, Green H.(1965). The initiation of cell division in a contact-inhibited mammalian cell line. J Cell Physiol.;66(3):325-33.

Tohyama M, Hanakawa Y, Shirakata Y, Dai X, Yang L, Hirakawa S, Tokumaru S, Okazaki H, Sayama K, Hashimoto K. (2009) IL-17 and IL-22 mediate IL-20 subfamily cytokine production in cultured keratinocytes via increased IL-22 receptor expression. Eur J Immunol.;39(10):2779-88

Trifari S, Spits H.(2010).IL-22-producing CD4+ T cells: middle-men between the immune system and its environment. Eur J Immunol.;40(9):2369-71.

Trigg CJ, Manolitsas ND, Wang J, Calderón MA, McAulay A, Jordan SE, Herdman MJ, Jhalli N, Duddle JM, Hamilton SA, et al.(1994). Placebo-controlled immunopathologic study of four months of inhaled corticosteroids in asthma. Am J Respir Crit Care Med.;150(1):17-22

Truong-Tran AQ, Grosser D, Ruffin RE, Murgia C, Zalewski PD.(2003). Apoptosis in the normal and inflamed airway epithelium: role of zinc in epithelial protection and procaspase-3 regulation. Biochem Pharmacol.;66(8):1459-68

van de Pol MA, Lutter R, van Ree R, van der Zee JS.(2012). Increase in allergen-specific IgE and ex vivo Th2 responses after a single bronchial challenge with house dust mite in allergic asthmatics. Allergy.;67(1):67-73

Van Maele L, Carnoy C, Cayet D, Songhet P, Dumoutier L, Ferrero I, Janot L, Erard F, Bertout J, Leger H, Sebbane F, Benecke A, Renauld JC, Hardt WD, Ryffel B, Sirard JC.(2010). TLR5 signaling stimulates the innate production of IL-17 and IL-22 by CD3(neg)CD127+ immune cells in spleen and mucosa. J Immunol.;185(2):1177-85

Vegiopoulos A, Herzig S.(2007)Glucocorticoids, metabolism and metabolic diseases.Mol Cell Endocrinol. 2007 Sep 15;275(1-2):43-61.

Veldhoen M, Stockinger B.(2006). TGFbeta1, a "Jack of all trades": the link with pro-inflammatory IL-17-producing T cells.Trends Immunol.;27(8):358-61

Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, Stockinger B.(2008).The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins.Nature.;453(7191):106-9

Veldhoen M, Hirota K, Christensen J, O'Garra A, Stockinger B.(2009).Natural agonists for aryl hydrocarbon receptor in culture medium are essential for optimal differentiation of Th17 T cells.J Exp Med.;206(1):43-9

Vendelin J, Pulkkinen V, Rehn M, Pirskanen A, Räisänen-Sokolowski A, Laitinen A, Laitinen LA, Kere J, Laitinen T.(2005). Characterization of GPRA, a novel G protein-coupled receptor related to asthma.Am J Respir Cell Mol Biol.;33(3):262-70

Veny M, Esteller M, Ricart E, Piqué JM, Panés J, Salas A. (2010) Late Crohn's disease patients present an increase in peripheral Th17 cells and cytokine production compared with early patients. Aliment Pharmacol Ther.;31(5):561-72

Vercelli D. (2005).Genetic regulation of IgE responses: Achilles and the tortoise.J Allergy Clin Immunol.;116(1):60-4

Vignola AM, Chanez P, Bonsignore G, Godard P, Bousquet J.(2000). Structural consequences of airway inflammation in asthma.J Allergy Clin Immunol.;105(2 Pt 2):S514-7

Vinuesa CG, Tangye SG, Moser B, Mackay CR.(2005). Follicular B helper T cells in antibody responses and autoimmunity.Nat Rev Immunol.;5(11):853-65

Volpe E, Touzot M, Servant N, Marloie-Provost MA, Hupé P, Barillot E, Soumelis V.(2009).Multiparametric analysis of cytokine-driven human Th17 differentiation reveals a differential regulation of IL-17 and IL-22 production. Blood.;114(17):3610-4

von Mutius E, Fritzsch C, Weiland SK, Röll G, Magnussen H (1992).Prevalence of asthma and allergic disorders among children in united Germany: a descriptive comparison.BMJ.;305(6866):1395-9

von Mutius E, Nicolai T.(1996).Familial aggregation of asthma in a South Bavarian population.Am J Respir Crit Care Med. 1996 Apr;153(4 Pt 1):1266-72.

Vrtala S, Hirtenlehner K, Susani M, Akdis M, Kussebi F, Akdis CA, Blaser K, Hufnagl P, Binder BR, Politou A, Pastore A, Vangelista L, Sperr WR, Semper H, Valent P, Ebner C, Kraft D, Valenta R. (2001) Genetic engineering of a hypoallergenic trimer of the major birch pollen allergen Bet v 1. FASEB J.;15(11):2045-7.

Wahl C, Wegenka UM, Leithäuser F, Schirmbeck R, Reimann J.(2009). IL-22-dependent

attenuation of T cell-dependent (ConA) hepatitis in herpes virus entry mediator deficiency. J Immunol.;182(8):4521-8

Wang HY, Wong GW, Chen YZ, Ferguson AC, Greene JM, Ma Y, Zhong NS, Lai CK, Sears MR.(2008). Prevalence of asthma among Chinese adolescents living in Canada and in China.CMAJ.;179(11):1133-42

Wang Y, Bai C, Li K, Adler KB, Wang X.(2008). Role of airway epithelial cells in development of asthma and allergic rhinitis. Respir Med.;102(7):949-55

Wang LF, Chiu HC, Hsu CJ, Liu CY, Hsueh YH, Miaw SC. (2009) Epicutaneous sensitization with a protein antigen induces Th17 cells. J Dermatol Sci.;54(3):192-7

Waser M, Michels KB, Bieli C, Flöistrup H, Pershagen G, von Mutius E, Ege M, Riedler J, Schram-Bijkerk D, Brunekreef B, van Hage M, Lauener R, Braun-Fahrländer C; PARSIFAL Study team.(2007). Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe.Clin Exp Allergy.;37(5):661-70

Weber GF, Schlautkötter S, Kaiser-Moore S, Altmayr F, Holzmann B, Weighardt H.(2007).Inhibition of interleukin-22 attenuates bacterial load and organ failure during acute polymicrobial sepsis.Infect Immun.;75(4):1690-7

Weger W, Hofer A, Wolf P, El-Shabrawi Y, Renner W, Kerl H, Salmhofer W.(2009) Common polymorphisms in the interleukin-22 gene are not associated with chronic plaque psoriasis. Exp Dermatol.;18(9):796-8

Wei CC, Ho TW, Liang WG, Chen GY, Chang MS. (2003) Cloning and characterization of mouse IL-22 binding protein. Genes Immun.;4(3):204-11

Wei L, Laurence A, Elias KM, O'Shea JJ.(2007). IL-21 is produced by Th17 cells and drives IL-17 production in a STAT3-dependent manner. J Biol Chem.;282(48):34605-10

Wei G, Wei L, Zhu J, Zang C, Hu-Li J, Yao Z, Cui K, Kanno Y, Roh TY, Watford WT, Schones DE, Peng W, Sun HW, Paul WE, O'Shea JJ, Zhao K.(2009). Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. Immunity.;30(1):155-67

Weiland SK, von Mutius E, Hirsch T, Duhme H, Fritzsch C, Werner B, Hüsing A, Stender M, Renz H, Leupold W, Keil U (1999). Prevalence of respiratory and atopic disorders among children in the East and West of Germany five years after unification. Eur Respir J. ;14(4):862-70

Wenzel S, Wilbraham D, Fuller R, Getz EB, Longphre M.(2007)Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies.Lancet;370(9596):1422-31.

Wenzel S.(2012)Severe asthma: from characteristics to phenotypes to endotypes.Clin Exp Allergy. ;42(5):650-8.

Whittington HA, Armstrong L, Uppington KM, Millar AB. (2004) Interleukin-22: a potential immunomodulatory molecule in the lung. Am J Respir Cell Mol Biol.;31(2):220-6

Wicks J, Haitchi HM, Holgate ST, Davies DE, Powell RM.(2006). Enhanced upregulation of smooth muscle related transcripts by TGF beta2 in asthmatic (myo) fibroblasts.

Thorax.;61(4):313-9

Wilson A, Pircher H, Ohashi P, MacDonald HR.(1992). Analysis of immature (CD4-CD8-) thymic subsets in T-cell receptor alpha beta transgenic mice. Dev Immunol.;2(2):85-94
Wilson CB, Rowell E, Sekimata M.(2009). Epigenetic control of T-helper-cell differentiation.Nat Rev Immunol.;9(2):91-105

Wilson MS, Feng CG, Barber DL, Yarovinsky F, Cheever AW, Sher A, Grigg M, Collins M, Fouser L, Wynn TA.(2010).Redundant and pathogenic roles for IL-22 in mycobacterial, protozoan, and helminth infections. J Immunol.;184(8):4378-90

Wilson NJ, Boniface K, Chan JR, McKenzie BS, Blumenschein WM, Mattson JD, Basham B, Smith K, Chen T, Morel F, Lecron JC, Kastelein RA, Cua DJ, McClanahan TK, Bowman EP, de Waal Malefyt R.(2007). Development, cytokine profile and function of human interleukin 17-producing helper T cells.Nat Immunol.;8(9):950-7

Wolk K, Kunz S, Asadullah K, Sabat R.(2002). Cutting edge: immune cells as sources and targets of the IL-10 family members? J Immunol.;168(11):5397-402

Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R.(2004). IL-22 increases the innate immunity of tissues. Immunity.;21(2):241-54

Wolk K, Witte E, Reineke U, Witte K, Friedrich M, Sterry W, Asadullah K, Volk HD, Sabat R.(2005). Is there an interaction between interleukin-10 and interleukin-22? Genes Immun.;6(1):8-18

Wolk K, Sabat R.(2006). Interleukin-22: a novel T- and NK-cell derived cytokine that regulates the biology of tissue cells.Cytokine Growth Factor Rev.;17(5):367-80

Wolk K, Witte E, Wallace E, Döcke WD, Kunz S, Asadullah K, Volk HD, Sterry W, Sabat R.(2006). IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis.Eur J Immunol.;36(5):1309-23

Wolk K, Witte E, Hoffmann U, Doecke WD, Endesfelder S, Asadullah K, Sterry W, Volk HD, Wittig BM, Sabat R.(2007). IL-22 induces lipopolysaccharide-binding protein in hepatocytes: a potential systemic role of IL-22 in Crohn's disease.J Immunol. ;178(9):5973-81

Wolk K, Haugen HS, Xu W, Witte E, Waggie K, Anderson M, Vom Baur E, Witte K, Warszawska K, Philipp S, Johnson-Leger C, Volk HD, Sterry W, Sabat R. (2009). IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-gamma are not. J Mol Med (Berl).;87(5):523-36.

Wolk K, Witte E, Warszawska K, Schulze-Tanzil G, Witte K, Philipp S, Kunz S, Döcke WD, Asadullah K, Volk HD, Sterry W, Sabat R.(2009).The Th17 cytokine IL-22 induces IL-20 production in keratinocytes: a novel immunological cascade with potential relevance in psoriasis. Eur J Immunol.;39(12):3570-81

Wong CK, Ho CY, Ko FW, Chan CH, Ho AS, Hui DS, Lam CW.(2001).Proinflammatory cytokines (IL-17, IL-6, IL-18 and IL-12) and Th cytokines (IFN-gamma, IL-4, IL-10 and IL-13) in patients with allergic asthma. Clin Exp Immunol. ;125(2):177-83

Wong GW, Chow CM.(2008). Childhood asthma epidemiology: insights from comparative studies of rural and urban populations.Pediatr Pulmonol.;43(2):107-16

Wong CK, Lun SW, Ko FW, Wong PT, Hu SQ, Chan IH, Hui DS, Lam CW.(2009). Activation of peripheral Th17 lymphocytes in patients with asthma.Immunol Invest.;38(7):652-64

Wood RA, Phipatanakul W, Hamilton RG, Eggleston PA. (1999) A comparison of skin prick tests, intradermal skin tests, and RASTs in the diagnosis of cat allergy. J Allergy Clin Immunol.;103(5 Pt 1):773-9

Wu PW, Li J, Kodangattil SR, Luxenberg DP, Bennett F, Martino M, Collins M, Dunussi-Joannopoulos K, Gill DS, Wolfman NM, Fouser LA.(2008).IL-22R, IL-10R2, and IL-22BP binding sites are topologically juxtaposed on adjacent and overlapping surfaces of IL-22. J Mol Biol. ;382(5):1168-83.

Xie MH, Aggarwal S, Ho WH, Foster J, Zhang Z, Stinson J, Wood WI, Goddard AD, Gurney AL.(2000). Interleukin (IL)-22, a novel human cytokine that signals through the interferon receptor-related proteins CRF2-4 and IL-22R.J Biol Chem.;275(40):31335-9

Xu T, Logsdon NJ, Walter MR.(2005). Structure of insect-cell-derived IL-22. Acta Crystallogr D Biol Crystallogr.;61(Pt 7):942-50

Xu W, Presnell SR, Parrish-Novak J, Kindsvogel W, Jaspers S, Chen Z, Dillon SR, Gao Z, Gilbert T, Madden K, Schlutsmeyer S, Yao L, Whitmore TE, Chandrasekher Y, Grant FJ, Maurer M, Jelinek L, Storey H, Brender T, Hammond A, Topouzis S, Clegg CH, Foster DC.(2001). A soluble class II cytokine receptor, IL-22RA2, is a naturally occurring IL-22 antagonist. Proc Natl Acad Sci U S A. ;98(17):9511-6

Yang XO, Panopoulos AD, Nurieva R, Chang SH, Wang D, Watowich SS, Dong C.(2007). STAT3 regulates cytokine-mediated generation of inflammatory helper T cells.J Biol Chem.;282(13):9358-63

Yang IA, Fong KM, Zimmerman PV, Holgate ST, Holloway JW.(2009). Genetic susceptibility to the respiratory effects of air pollution. Postgrad Med J. 2009 Aug;85(1006):428-36

Yang Y, Lu JY-L, Wu X, Summer S, Whoriskey J, Saris C, Reagan JD (2010) G-Protein-Coupled Receptor 35 Is a Target of the Asthma Drugs Cromolyn Disodium and Nedocromil Sodium;Pharmacology ;86:1–5

Yang L, Zhang Y, Wang L, Fan F, Zhu L, Li Z, Ruan X, Huang H, Wang Z, Huang Z, Huang Y, Yan X, Chen Y. (2010b) Amelioration of high fat diet induced liver lipogenesis and hepatic steatosis by interleukin-22. J Hepatol. Aug;53(2):339-47

Yao S, Huang D, Chen CY, Halliday L, Zeng G, Wang RC, Chen ZW.(2010). Differentiation, distribution and gammadelta T cell-driven regulation of IL-22-producing T cells in tuberculosis. PLoS Pathog. 26;6(2):e1000789

Yeh JH, Sidhu SS, Chan AC.(2008). Regulation of a late phase of T cell polarity and effector functions by Crtam.Cell.;132(5):846-59

Ying S, Robinson DS, Meng Q, Rottman J, Kennedy R, Ringler DJ, Mackay CR, Daugherty BL, Springer MS, Durham SR, Williams TJ, Kay AB.(1997). Enhanced expression of eotaxin and CCR3 mRNA and protein in atopic asthma. Association with airway hyperresponsiveness and predominant co-localization of eotaxin mRNA to bronchial epithelial and endothelial cells.Eur J Immunol.;27(12):3507-16

Zanini A, Chetta A, Imperatori AS, Spanevello A, Olivieri D.(2001). The role of the bronchial microvasculature in the airway remodelling in asthma and COPD.Respir Res.;11:132

Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Karow M, Flavell RA. (2007) Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. Immunity.;27(4):647-59

Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Stevens S, Flavell RA.(2008). Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease.Immunity.;29(6):947-57

Zhang K, Rekhter MD, Gordon D, Phan SH.(1994). Myofibroblasts and their role in lung collagen gene expression during pulmonary fibrosis. A combined immunohistochemical and in situ hybridization study. Am J Pathol.;145(1):114-25

Zhang X, Jin J, Peng X, Ramgolam VS, Markovic-Plese S.(2008). Simvastatin inhibits IL-17 secretion by targeting multiple IL-17-regulatory cytokines and by inhibiting the expression of IL-17 transcription factor RORC in CD4+ lymphocytes. J Immunol.;180(10):6988-96

Zhang Z, Luo YL, Zhou LL, Lai WY, Xu J, Li ZX, Ren DQ, Ye H, Zhong HH. (2011) Change of IL-22R1 expression in human airway smooth muscle cells in response to different stimulating agents. Nan Fang Yi Ke Da Xue Xue Bao.;31(6):979-82

Zhang L, Li YG, Li YH, Qi L, Liu XG, Yuan CZ, Hu NW, Ma DX, Li ZF, Yang Q, Li W, Li JM.(2012). Increased frequencies of Th22 cells as well as Th17 cells in the peripheral blood of patients with ankylosing spondylitis and rheumatoid arthritis.PLoS One.;7(4):e31000

Zhao Y, Yang J, Gao YD, Guo W. (2010) Th17 immunity in patients with allergic asthma. Int Arch Allergy Immunol.;151(4):297-307

Zheng W, Flavell RA.(1997). The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell.;89(4):587-96

Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, Ouyang W.(2007). Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis.Nature.;445(7128):648-51

Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q, Abbas AR, Modrusan Z, Ghilardi N, de Sauvage FJ, Ouyang W.(2008). Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens.Nat Med.;14(3):282-9

Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR.(2007). IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat Immunol.;8(9):967-74

Zhu J, Cote-Sierra J, Guo L, Paul WE.(2003). Stat5 activation plays a critical role in Th2 differentiation. Immunity.;19(5):739-48

Zhu J, Min B, Hu-Li J, Watson CJ, Grinberg A, Wang Q, Killeen N, Urban JF Jr, Guo L, Paul WE.(2004). Conditional deletion of Gata3 shows its essential function in T(H)1-T(H)2 responses.Nat Immunol.;5(11):1157-65

Zhu J, Cao Y, Li K, Wang Z, Zuo P, Xiong W, Xu Y, Xiong S.(2011). Increased expression of aryl hydrocarbon receptor and interleukin 22 in patients with allergic asthma.Asian Pac J

Allergy Immunol.;29(3):266-72

Zock JP, Heinrich J, Jarvis D, Verlato G, Norbäck D, Plana E, Sunyer J, Chinn S, Olivieri M, Soon A, Villani S, Ponzio M, Dahlman-Hoglund A, Svanes C, Luczynska C; Indoor Working Group of the European Community Respiratory Health Survey II.(2006). Distribution and determinants of house dust mite allergens in Europe: the European Community Respiratory Health Survey II.J Allergy Clin Immunol.;118(3):682-90

Appendix

Additional experimental figures

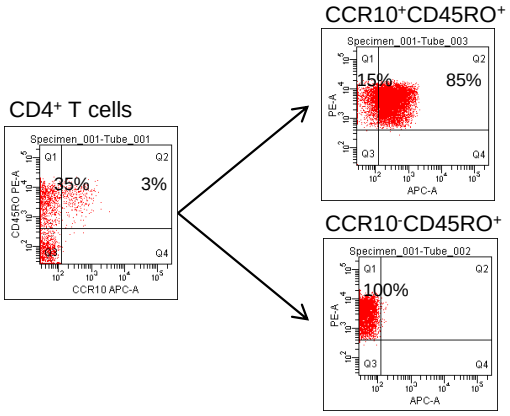


Figure 50. Flow cytometric analysis of flow-assisted-cell-sorted CCR10^{+} and CCR10^{-} CD45RO^{+} CD4^{+} T cells

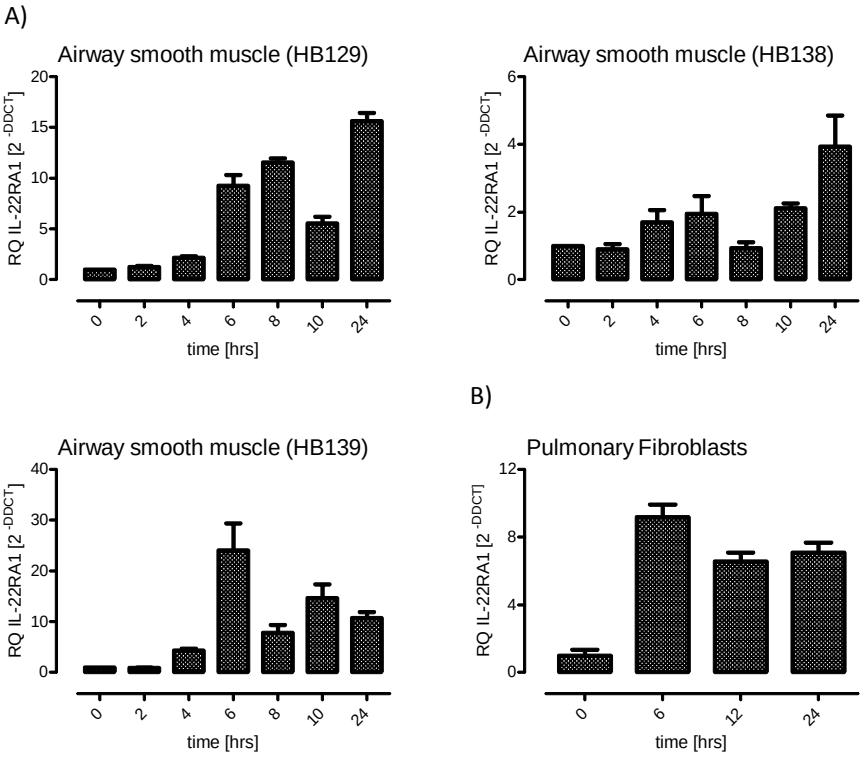


Figure 51. RT-PCR analysis of the induction of IL-22RA1 mRNA by IFNγ in airway smooth muscle cells and fibroblasts over time

A-C) Normal human airway smooth muscle B) Normal human pulmonary fibroblasts. Sample population n=3; Statistical test (wilcoxon)

Raw data tables

ID	Age (Yrs)	Gender (M/F)	Treatment Group	PC20 (mg/ml)	SPT ≥3mm	Total IgE (IU/ml)	RAST (IU/ml) Challenged Allergen	Challenged Allergen	FEV1-%predicted (before challenge)	LRP (% fall FEV1)
Sv01	24	F	Allergic Asthmatic	4	HDM, TG	448	12.3	HDM	132	39
Sv02	24	F	Allergic Asthmatic	1.8	HDM, Cat	388	54.4	HDM	119	22
Sv03	43	F	Allergic Asthmatic	2.75	HDM, TG	155	0.84	HDM	89	23
Sv04	33	F	Allergic Asthmatic	7	HDM, TG	319	23.1	HDM	116	31
Sv05	43	F	Allergic Asthmatic	15	TG	564	100	TG	96	33
Sv06	41	F	Allergic Asthmatic	1.2	TG	86	33	TG	90	21
Sv07	22	M	Allergic Asthmatic	25	TG	129	45.9	TG	105	8
Sv08	24	F	Allergic Asthmatic	32	TG	210	100	TG	88	12
Sv09	33	M	Allergic Asthmatic	2	TG, TP	538	44.2	TG	115	19
Sv10	38	M	Allergic Asthmatic	0.75	HDM	895	100	HDM	93	28
Sv11	25	M	Allergic Asthmatic	0.62	TG, TP	118	39.5	TG	138	25
Sv12	30	F	Allergic Asthmatic	4	HDM, TG	106	25.6	TG	97	41
Sv13	30	M	Allergic Asthmatic	1.5	TG	90	32.9	TG	145	24
Sv15	44	F	Allergic Asthmatic	16	TG	340	101	TG	105	36
Sv16	34	M	Allergic Asthmatic	0.9	TG	989	100	TG	119	18
AC16	25	M	Normal Control	>32	None	20	<0.35	HDM	>100	8
AC18	n.a.	n.a.	Normal Control	>32	None	n.a.	<0.35	HDM	>100	8
AC19	25	M	Normal Control	>32	None	n.a.	<0.35	HDM	>100	8
AC23	n.a.	n.a.	Normal Control	>32	None	n.a.	<0.35	HDM	>100	8
AC26	19	M	Normal Control	>32	None	46	<0.35	HDM	>100	8

ID	Eos (10^6) before	Eos (10^6) after	IL-5 (pg/ml) before	IL-5 (pg/ml) after	IFNγ (pg/ml) before	IFNγ (pg/ml) after	IL-17A/F (pg/ml) before	IL-17A/F (pg/ml) after	IL-22 (pg/ml) before	IL-22 (pg/ml) after	IL-22BP (ng/ml) before	IL-22BP (ng/ml) after
Sv01	0.094	0.771	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0
Sv02	0.142	13.3	<15.0	311.8	<15.0	<15.0	<15.0	<15.0	<15.0	117.2	<15.0	53526.4
Sv03	n.a.	n.a.	<15.0	<15.0	15.44	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	96.8
Sv04	0	2.304	<15.0	15.7	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0
Sv05	0	2.9	<15.0	15.9	<15.0	<15.0	<15.0	<15.0	<15.0	22.8	n.a.	n.a.
Sv06	0	2.9	<15.0	21.2	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	n.a.	n.a.
Sv07	0.45	6.282	<15.0	971.6	<15.0	<15.0	<15.0	<15.0	<15.0	54.3	<15.0	1767.5
Sv08	0.1	0.6	<15.0	18.5	<15.0	<15.0	<15.0	<15.0	<15.0	18.4	n.a.	n.a.
Sv09	0	2.1	<15.0	25.6	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	n.a.	n.a.
Sv10	0.05	0.6	<15.0	26.8	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0
Sv11	0	17.19	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0
Sv12	0.1	4.5	<15.0	26.9	<15.0	26.9	<15.0	<15.0	<15.0	15.3	n.a.	n.a.
Sv13	0	5.2	<15.0	41.7	<15.0	<15.0	<15.0	<15.0	<15.0	50.9	45.2	15343.8
Sv15	0	14.69	<15.0	91.9	<15.0	18.89	<15.0	<15.0	<15.0	32.9	<15.0	15298.0
Sv16	0	80.01	<15.0	444.8	<15.0	<15.0	<15.0	<15.0	<15.0	19.3	<15.0	16.5
AC16	0.07	0.08	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0
AC18	0.0	0.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
AC19	0.1	0.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
AC23	n.a.	n.a.	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0
AC26	n.a.	n.a.	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0	<15.0

Table 67. Individual clinical data as well as experimental results of the bronchial allergen challenge study

HDM= House Dust Mite, TG= Timothy TG (*Phleum pratense*), TP= Tree Pollen.

Sample ID	Age (Yrs)	Gender (M/F)	Treatment Group	Challenged Allergen	Skin Prick Test*	Additional SPT+	Total IgE (IU/ml)	RAST (IU/ml)	LPR (8hrs) diluent (mm2)	LPR (8hrs) allergen (mm2)	Eosinophils diluent (cells/mm2)	Eosinophils allergen (cells/mm2)
G1	28	M	Atopic	TG	14	C	1570	101	407	6575.8	0	346.5
G2	30	F	Atopic	TG	5	C, V	671	14	471	4140	0	347.5
G3	49	M	Atopic	TG	5	No	34	3.14	223.5	5380	19.5	194.5
G5	21	M	Atopic	TG	11	B, T	736	101	545	5680	0	136.1
G6	21	M	Atopic	TG	7	M, D	308	101	381.5	5308	2.5	293
G7	53	M	Atopic	TG	5	No	41	3.38	390.5	4670.3	2.7	240
G8	25	F	Atopic	TG	10	D	135	53.5	316	5501.5	8.5	350
G9	26	M	Atopic	TG	8	No	46	12.9	332	4459	1.1	224
G10	50	M	Atopic	TG	9	No	28	10.7	0	3222.8	0	262.5
G11	28	M	Atopic	TG	7	No	25	14.3	0	3094.3	0	155.5
G13	25	M	Atopic	TG	9	M, T, B, C	615	82.3	0	3854.3	3.1	330.7
G14	29	F	Atopic	TG	8	M, T, C, D, H	207	50.8	0	3352	0	63.8
G15	27	F	Atopic	TG	11	T, B, C, H	1017	69.3	0	3130	1.3	174.4
G17	27	M	Atopic	TG	10	No	83	29.3	0	3357	0	133
N1	29	F	Normal	TG	0	No	11	0.34	0	0	0	1.9
N2	22	F	Normal	TG	0	No	16	0.34	0	0	0	2.5
N3*	29	M	Normal	TG	2	No	853	2.11	0	0	0	0
N4	33	F	Normal	TG	0	No	28	0.42	0	0	0	4.5
N5	26	M	Normal	TG	0	No	3	0.34	0	0	0	39.4
N6	41	F	Normal	TG	0	No	24	0.34	0	0	10	0

Table 68. Individual clinical data as well as experimental results of the intradermal allergen challenge study

* N3 was included in normal control group but excluded from analysis (high levels of total & specific IgE) ; *Skin Prick test against Grass; TG= Timothy Grass (*Phleum pratense*), C=Cat, B=Birch, T=Tree, M=?, D=?, H=House Dust Mite, V=?

Sample ID	Age (Y)	Gender (M/F)	Treatment Group	SPT ≥3mm	Multiple Allergens	Total IgE (IU/ml)	RAST (IU/ml)	IL-5 (pg/ml) control	IL-5 (pg/ml) 5µg/ml Phl P	IFNγ (pg/ml) control	IFNγ (pg/ml) 5µg/ml Phl P	IL-17A/F (pg/ml) control	IL-17A/F (pg/ml) Phl P	IL-22 (pg/ml) control	IL-22 (pg/ml) 5µg/ml Phl P
Tc184	30	F	Atopic	TG	Yes	157	18.7	<15.0	<15.0	33.62	34.21	<8.0	<8.0	<15.0	<15.0
Tc185	36	M	Atopic	TG	Yes	267	33.9	<15.0	362.6	<15.0	37.71	<8.0	<8.0	<15.0	56.9
Tc187	30	F	Atopic	TG	Yes	714	23.2	<15.0	32.4	<15.0	<15.0	<8.0	<8.0	<15.0	<15.0
Tc188	35	F	Atopic	TG	Yes	163	11.6	<15.0	214.5	<15.0	<15.0	<8.0	<8.0	<15.0	<15.0
Tc192	45	M	Atopic	TG	Yes	35	1.9	<15.0	353.7	26.63	27.7	<8.0	<8.0	<15.0	<15.0
Tc193	28	F	Atopic	TG	Yes	793	88.4	<15.0	<15.0	54.17	57.51	<8.0	12.2	<15.0	<15.0
BAA310	53	M	Atopic	TG	Yes	30	1.96	73.95	128.0	<15.0	<15.0	<8.0	<8.0	97.13	122.2
BAA311	30	F	Atopic	TG	Yes	714	23.2	32.42	31.07	<15.0	<15.0	<8.0	<8.0	<15.0	<15.0
11384	37	F	Atopic	TG	Yes	61.1	14.8	<15.0	46.07	24.59	22.31	<8.0	<8.0	<15.0	97.13
11620	29	M	Atopic	TG	No	16.3	7.74	<15.0	18.83	20.53	20.12	<8.0	<8.0	<15.0	<15.0
11994	24	F	Atopic	TG	No	290	>100	<15.0	83.2	19.51	19.51	<8.0	<8.0	<15.0	<15.0
12160	21	M	Atopic	TG	No	246	42.3	<15.0	83.9	19.61	19.92	<8.0	<8.0	<15.0	<15.0
Tc189	56	M	Normal	None	-	36	0.03	<15.0	<15.0	42.31	38.96	10.8	9..8	<15.0	99.9
Tc190	56	M	Normal	None	-	6	<0.01	<15.0	<15.0	<15.0	<15.0	<8.0	<8.0	<15.0	92.8
Tc191	23	M	Normal	None	-	105	<0.01	<15.0	37.6	<15.0	<15.0	<8.0	<8.0	<15.0	363.7
Tc195	36	M	Normal	None	-	21	0.01	<15.0	<15.0	30.35	29.16	<8.0	8.6	<15.0	25.3
Tc196	55	F	Normal	None	-	6	<0.01	<15.0	<15.0	<15.0	<15.0	<8.0	<8.0	<15.0	59.1
Tc197	56	F	Normal	None	-	26	0.01	<15.0	<15.0	<15.0	<15.0	<8.0	<8.0	<15.0	<15.0
Tc203	56	M	Normal	None	-	36	0.03	<15.0	<15.0	21.53	21.63	<8.0	<8.0	<15.0	<15.0
Tc204	42	M	Normal	None	-	5	0.01	<15.0	<15.0	21.73	20.63	<8.0	<8.0	<15.0	<15.0
Tc208	57	F	Normal	None	-	26	<0.01	<15.0	<15.0	21.23	20.23	<8.0	<8.0	<15.0	<15.0
Tc209	25	F	Normal	None	-	n.a.	n.a.	<15.0	<15.0	<15.0	<15.0	<8.0	<8.0	<15.0	<15.0
BNorm409	37	M	Normal	None	-	21	0.01	<15.0	<15.0	<15.0	<15.0	<8.0	<8.0	<15.0	<15.0

Table 69. Individual clinical data as well as experimental results of the *in vitro* allergen PBMC challenge study

TG= Timothy TG (*Phleum pratense*)

ID	Age (Yrs)	Gender (M/F)	Treatment Group	Asthma	Steroid use	Smoking History	Other Clinical Conditions	Skin Prick Test(1)	Total IgE (IU/ml)	RAST (IU/ml)	Bronchoscopy (Date)	Other ID Codes
B01	33	M	Allergic Asthma	Severe	Prednisolone	Never smoked	Eczema, Hayfever	Positive	3016	100 (HDM), 28(GP), 95(DOG),5.5(CAT)	02/12/2009	MAPK056 ASM14 OXI24
B02	37	F	Asthma	Severe	None	Never smoked	Sinusitis, Gout	Negative	315	<0.35 (all)	03/02/2010	MAPK057 ASM16 OXI26
B03	42	M	Asthma	Severe	Prednisolone	Never smoked	Osteopenia	Negative	15	<0.35 (all)	17/02/2010	MAPK066 OXI32
B04	37	M	Allergic Asthma	Mild/Moderate	None	Never smoked	Eczema, Hayfever	Positive	845	*Not available*	24/02/2010	ASM017
B05	57	F	Asthma	Severe	None	Never smoked	Pulmonary eosinophilia	Negative	153	<0.35 (all)	14/04/2010	OXI40 MAPK016 ASM26
B06	35	M	Asthma	Mild/Moderate	None	Never smoked	Sinusitis,Spienectomy	Negative	182	<0.35 (all)	05/05/2010	MAPK074 ASM24 OXI38

Table 70. Individual data of patients taking part in the study of T cell lines and clones derived from asthmatic bronchial mucosal tissue

(1) The Skin Prick Test was performed for several allergens (dog, cat, house dust mite, and aspergillus fumigatus). RAST Test results for B04 were not available.