Department of Medicine Solna, Respiratory Medicine Unit
Karolinska Institutet, Stockholm, Sweden

Lung T cells in inflammatory disorders

An Approach to Interstitial Lung Disease, Multiple Sclerosis and Smoking Induced Inflammation

Mahyar Ostadkarampour

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Cover photo by Mahyar Ostadkarampour. Fluorospot analysis of IFNγ and IL-17 producing cells from BAL of sarcoidosis patient. The green and red spots indicate IFNγ and IL-17 producing cells respectively after anti CD3 stimulation. The yellow circles show the cells that produce both cytokines simultaneously. The experiment was analyzed by an automated reader with filters for FITC and Cy3 in Mabtech company.

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Lung T cells in inflammatory disorders, An Approach to Interstitial Lung Disease, Multiple Sclerosis and Smoking Induced Inflammation

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Mahyar Ostadkarampour

Principal Supervisor:
Associate professor Jan Wahlström
Karolinska Institutet
Department of Medicine Solna
Respiratory Medicine Unit

Co-supervisor(s):
Professor Johan Grunewald
Karolinska Institutet
Department of Medicine Solna
Respiratory Medicine Unit

Opponent:
Associate professor Kristina Lejon
Umeå University
Department of Clinical Microbiology

Examination Board:
Associate professor Mikael Adner
Karolinska Institutet
Department of Institute of Environmental Medicine

Associate professor Rosaura Casas
Linköping University
Department of Clinical and Experimental Medicine

MD PhD Liv Eidsmo
Karolinska Institutet
Department of Medicine, Solna

To Behnaz and Baran

ABSTRACT

The lungs are constantly exposed to microorganisms and environmental irritants. Pulmonary inflammation is the result of an immune process to protect the body, and may sometimes eventually result in disease. T cells including various subsets are of major importance for orchestrating the protection of the lung as well as for inflammatory reactions. Activated pulmonary T cells not only have the potential to affect the lungs themselves, but they could contribute to immune responses in other organs as well. The overall aim of the study presented in this thesis was to investigate the potential effect of T cell immune responses for chronic lung inflammation from two different aspects. We thus first investigated antigen-specific T cell responses in patients with pulmonary sarcoidosis, and in the second part determined how smoking affected lung T-cell immunity, with regard to the influence of smoking in the development of autoimmunity.

Sarcoidosis is a granulomatous systemic inflammatory disorder which commonly affects the lungs. T cells and particularly activated CD4+ T cells are considered to be involved in the pathogenesis of the disease. A subgroup of sarcoidosis patients known as Löfgren's syndrome differs strikingly from other patients by particular clinical symptoms. Spontaneous recovery within two years is particularly common in Löfgren´s syndrome patients who are HLA-DRB1*0301positive, and these patients virtually always have accumulations of T cells expressing a particular T cell receptor (TCR) V gene segment, termed AV2S3, in the lungs. The aetiology of sarcoidosis is still not known. However, recently a specific mycobacterial protein, *M. tuberculosis* catalase-peroxidase (mKatG) was identified in sarcoidosis tissues.

BAL CD4+ T cells from HLA-DRB1*0301positive Löfgren's syndrome responded to mKatG with a more pronounced multifunctional cytokine profile, i.e. with simultaneous production of IFNγ and TNF compared to non-Löfgren's syndrome patients. Non-Löfgren's syndrome patients instead responded with a higher proportion of cells producing single cytokines, i.e. production of IFNγ alone. Moreover, AV2S3+ CD4+ T cells from both BAL and blood had a higher IFNγ production in response to mKatG compared to AV2S3- CD4+ T cells, while the opposite was found for BAL AV2S3+ CD4+ cells in response to PPD. Furthermore, BAL T cells from Löfgren´s syndrome patients had compared to T cells of non Löfgren´s syndrome higher frequencies of IL-17-producing cells in response to mKatG. Löfgren´s syndrome HLA-DRB1*03 positive patients clearly had higher levels of IL-17 in BAL fluid compared to healthy controls and to patients without Löfgren's syndrome. Our results indicate that the quality of the T cell response in sarcoidosis patients may play a key role in disease presentation and clinical outcome. These findings imply that the presence of multifunctional BAL CD4+ T cells, higher activities of TCR AV2S3+ CD4+ T cells, and more pronounced IL-17 production in particular subgroups of sarcoidosis patients are involved in antigen elimination at the site of inflammation and may play a role in spontaneous recovery, typical for patients with Löfgren´s syndrome (in particular DRB1*03 positive).

Cigarette smoking is a well-known risk factor for several inflammatory and autoimmune disorders. The risk of developing multiple sclerosis (MS) is strongly increased by smoking in people with genetic susceptibility. Smoking is associated with both release and inhibition of pro-inflammatory and anti-inflammatory mediators that influence different T cell subsets. Our results indicate that cigarette smoke induces a decline in lung Th17 cells and alters the phenotype of T regulatory cells by decreasing the proportion of IL-10 producing Foxp3+ CD4+ cells and increasing the fraction of lung Foxp3+ Helios negative T cells. Thus, an imbalance between Th17/Tregs may be caused by cigarette smoking, which could result in an increased risk for infection and may also have consequences for autoimmune processes postulated to be initiated in the lung. Furthermore we studied the effect of smoking and conventional treatment in the lungs and blood of MS patients compared to healthy individuals. We found that the frequency of Foxp3+Helios+ regulatory T cells, important in the context of autoimmunity, was reduced in BAL of MS patients. However, the frequencies of both this subset of Tregs and of total Foxp3+ CD4+ BAL Treg cells was increased after treatment particularly in IFNβ treated MS patients. If the lungs are involved in initiation and propagation of inflammatory processes in MS, the observed effects in IFNβ-treated patients may be involved in disease amelioration in MS patients following such treatment.

LIST OF SCIENTIFIC PAPERS

I. Maria Wikén, Mahyar Ostadkarampour, Anders Eklund, Matthew Willett, Edward Chen, David Moller, Johan Grunewald, Jan Wahlström; Antigen-specific multifunctional T-cells in sarcoidosis patients with Löfgren's syndrome; he European Respiratory Journal, 2012 Jul;40(1):110-21

II. Mahyar Ostadkarampour, Anders Eklund, David Moller, Pernilla Glader, Caroline Olgart Höglund, Anders Lindén, Johan Grunewald, Jan Wahlström; Higher levels of IL-17 and antigen-specific IL-17 responses in pulmonary sarcoidosis patients with Löfgren's syndrome; Clin Exp Immunol. 2014 Nov;178(2):342-52. doi: 10.1111/cei.12403.

III. Mahyar Ostadkarampour, Malin Müller, Johan Öckinger, Susanna Kullberg, Anders Eklund, Johan Grunewald, Jan Wahlström; Cigarette smoke induces distinctive Tregs and a Treg/Th17 imbalance in the lungs (Submitted)

IV. Mahyar Ostadkarampour, Susanna Kullberg, Johan Öckinger, Anders Eklund, Fredrik Piehl, Tomas Olsson, Johan Grunewald, Jan Wahlström; Characteristics of lung T cell subsets in multiple sclerosis patients with respect to smoking and beta-interferon treatment (Manuscript)

CONTENTS

LIST OF ABBREVIATIONS

ACPA	Anti citrullinated protein antibody
AHR	Aryl hydrocarbon receptor
AM	Alveolar macrophage
APC	Antigen presenting cell
AV2S3	Variable gene segment 2.3 of the T cell receptor α chain
BAL	Bronchoalveolar lavage
BCR	B cell receptor
BFA	Brefeldin A
BHL	Bilateral hilar lymphadenopathy
CBD	Chronic beryllium disease
CCR	Chemokine receptor
CD	Cluster of differentiation
CDR	Complementarity determining region
COPD	Chronic obstructive pulmonary disease
CTLA-4	Cytotoxic T lymphocyte-associated antigen 4
DAMP	Damage Associated Molecular Pattern
DC	Dendritic cell
DL_{CO}	Diffusing capacity of the lung for carbon monoxide
EAE	Experimental autoimmune encephalomyelitis
EAU	Experimental autoimmune uveitis
FACS	Fluorescence-activated cell sorter
FEV1	Forced expiratory volume in 1 second
Foxp3	Forkhead box protein 3
FVC	Forced vital capacity
GITR	Glucocorticoid-induced TNF receptor family
HLA	Human leukocyte antigen
IBD	Inflammatory bowel disease
ICAM-1	Intercellular adhesion molecule-1
IDO	Indoleamine 2,3-dioxygenase
IFNγ	Interferon-γ
Ig	Immunoglobulin
IL	Interleukin
ILD	Interstitial lung disease
iNKT	invariant natural killer T cell
IPF	Idiopathic pulmonary fibrosis

iTreg	Induced regulatory T cell
LPS	Lipopolysaccharide
MAIT	Mucosa-associated invariant T cells
MFI	Median (or mean) fluorescence intensity
MHC	Major histocompatibility complex
mKatG	Mycobacterial catalase-peroxidase
MS	Multiple sclerosis
NLR	NOD like receptor (nucleotide-binding oligomerization domain)
NK	Natural killer
PAMP	Pathogen associated molecular pattern
PBL	Peripheral blood lymphocytes
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PHA	Phytohaemagglutinin
PPD	Purified protein derivative
PRR	Pattern recognition receptor
pTreg	Peripheral regulatory T cell
qIPCR	quantitative immuno-PCR
RA	Rheumatoid Arthritis
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SEA	*Staphylococcus enterotoxin A*
SEB	*Staphylococcus enterotoxin B*
STAT	Signal Transducer and Activator of Transcription
TCR	T cell receptor
Tfh	Follicular helper T cell
TGFβ	Transforming growth factor-β
tTreg	Thymus-derived regulatory T cell
Th cell	T helper cell
TLR	Toll-like receptor
TNF	Tumor necrosis factor

1 INTRODUCTION

1.1 General introduction

Inflammation is a biological process with accumulation of leukocytes and dissemination of fluid and proteins in the inflamed tissue in response to infection, irritation, injury or physical stress. Acute inflammation is an important immune response to protect against pathogens. The inflammation usually clears after pathogen elimination, while if e.g. autoimmunity is induced or a pathogen persists there may be chronic inflammation which may lead to tissue destruction or fibrosis. Inflammation is an essential component of many lung disorders. T helper lymphocytes have a pivotal role in orchestrating the inflammatory reaction following triggering by specific antigens. In this thesis my aim is to better understand the role of T-cell immunity in pulmonary inflammation by focusing on the inflammatory interstitial lung disease sarcoidosis and by studying the effect of smoking on lung T cells with regard to the influence of smoking in developing autoimmune disease.

1.2 The immune system

The immune system protects the host from a variety of pathogens including bacteria, viruses and parasites and is also activated by stressed cells like malignant cells or cells with damaged DNA. Innate immunity provides the first line and initial immune responses against pathogens, while adaptive immunity consists of highly specialized immune responses against pathogen specific antigens.

1.3 Innate immunity

Innate immunity is a complex of early response mechanisms that provide the first-line defense to control and eliminate pathogens. The innate cells eradicate pathogens by rapid phagocytosis or release of killing substances; moreover they induce delayed cellular responses by activating signaling pathways that promote recruitment of more effector cells.

Innate immunity components

The first line of defense by the innate immunity system consists of physical (epithelial) or chemical (specialized substances) barriers. There are several substances (mostly proteins) which can protect the body from pathogens. Lysozyme, surfactants and defensins are examples of substances which are found in mucosal epithelia and the respiratory tract and that protect the body from invading pathogens by different mechanisms. If pathogens evade these defenses, then they encounter the cellular responses of innate immunity.

The cellular components of innate immunity react against the invading pathogens via receptors named pattern recognition receptors (PRR). PRR can recognize special structures on pathogens or stressed cells, but not normal host cells. These special structures which are common to many different pathogens or damaged cells are named pathogen associated molecular patterns (PAMP) or damage associated molecular patterns (DAMP).

PRRs are receptors which are expressed on the cell surface or intracellularly by a variety of cells and they contribute in activation of innate immunity. Toll like receptors (TLR), NOD like receptors (NLR) and Retinoic acid-inducible gene-I-like receptors (RLR) are examples of PRRs that can recognize many types of pathogen molecules. Examples of such lignds are

lipopolysaccharide (LPS) or peptidoglycan (presented by gram negative and gram positive bacteria respectively), double stranded RNA and unmethylated CpG DNA (1).

Phagocytic cells including neutrophils and macrophages engulf pathogens which are subsequently destroyed inside the phagosome. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are two well-studied digestive mechanisms which have the ability to destroy phagosome contents. NADPH oxidase and inducible nitric oxide synthase (iNOS) are two important enzymes that generate bactericidal ROS and NO respectively. These enzymes are activated when pathogens bind to PRRs. Phagosome fusion with lysosomes containing proteolytic protease enzymes is another important mechanism which helps to destroy ingested pathogens (2).

Natural Killer cells (NK cells) are a class of lymphoid cells which react against pathogens and abnormal cells. Lack of MHC class I, often induced by viral infection of a cell, is a signal for activation of these efficient killer cells to secrete cytotoxic granules. Moreover, NK cells by secretion of cytokines help to activate other immune cells (3).

In addition, there are several unconventional lymphocyte subsets that are classified as parts of innate immunity. Cells like γδ T cells, invariant natural killer T (iNKT) cells, Mucosa-associated invariant T cells (MAIT) and B-1 cells are lymphocytes that are activated in response to pathogens immediately when they recognize molecular patterns and have the capacity to immediately express effector functions (4).

1.4 Adaptive immunity

The second form of immunity that protects the host from invading pathogens is adaptive immunity. In contrast to innate immunity which responds to shared structures of pathogens in the early phase of infection, the function of adaptive immunity is defined by highly antigen specific responses against pathogens. Adaptive immunity responses develop later in the secondary phase to recognize and eliminate the pathogens more specifically and efficiently. The receptors in innate immunity are encoded by the germline DNA pattern, while in order to make an antigen specific response in adaptive immunity, receptors are encoded by gene segments that undergo somatic recombination to generate an enormous diversity of receptors. Immunologic memory is one important feature of adaptive immunity that allows more effective and rapid responses to occur during the second exposure to the antigens that the immune cells encountered before. The adaptive consists of B and T lymphocytes.

1.4.1 B cells

B cells are lymphocytes that mature in the bone marrow. Antibody production is the most prominent feature of B cells. B cells express immunoglobulin on their cell surface as the B cell receptor (BCR). Once B cells with cell surface immunoglobulin bind to specific matched antigens, they can internalize the antigen via endocytosis. B cells start to process internalized proteins into peptides and consequently they present antigenic peptides on their surface by MHC class II molecules to CD4+ T cells. If the T cell via its TCR recognizes the presented antigen it can provide help for B cell maturation. This response of B cells to antigens is classified as a T dependent response. B cells can also recognize non protein antigens via immunoglobulin without T cell involvement, which is called a T independent response. Activated B cells can differentiate into plasma cells that secrete soluble BCRs in the form of different classes of antibody. Secreted antibodies can protect the host by different

mechanisms. Antibodies help to destroy or inactivate the pathogens by neutralization (binding to pathogens can inhibit them from infecting other cells), opsonization (binding to pathogens will facilitate the phagocytosis by phagocytes) and finally by complement activation (a complex of serum proteins that via a catalytic cascade form mediators that help to destroy pathogens or make them suitable for phagocytosis) (2).

Somatic recombination of the variable regions in the immunoglobulin heavy chain (V, D and J segment) and light chain (V and J segment) generates the diversity in BCR and antibodies (5). B cells initially produce IgM and IgD, while following interaction of B cells with T cells, class switching will happen and IgG, IgE and IgA are induced in a T cell mediated manner.

1.4.2 T cells

T cells are lymphocytes which are responsible for the so-called cell mediated immunity and they develop in the bone marrow followed by maturation in the thymus. T lymphocytes are divided into two main classes that are different in phenotype and function. T cells which carry the CD4 molecule on their cell surface are named helper T cells and they are involved in activating B cells and phagocytes to destroy the pathogens. The second class of T cells is named cytotoxic T cells; they carry the CD8 molecule on their surface and kill their target cells. Despite differences between CD4 and CD8 T cells, they share common features. Naïve T cells can only recognize pathogen-derived peptides which are presented by antigen presenting cells (APC). Both CD4 and CD8 T cells have T cell receptors on their surface which are highly specific and recognize a unique antigen that is presented by special molecules on the surface of APCs. When naive T cells recognize appropriate antigen presented by APCs in lymphoid organs they will differentiate into effector cells which have the ability to react against pathogens and migrate to peripheral sites to eliminate pathogens. Generation of memory T cells following activation of T cells is one of the most important features of T cells (quite similar to B cells) leading to a faster and more effective response when the host encounters a secondary challenge with the same pathogen (6).

1.4.2.1 CD4+ T cells

CD4 T cells constitute a major subset of T cells that perform a pivotal role in the immune system. When naive CD4 T cells encounter their cognate antigen presented on APCs, they differentiate into effector and/or memory cells. CD4 T cells are named T helper cells because they have the ability to e.g. help B cells produce antibody. CD4 T cells help B cells to mount antibody responses, provide feedback to DCs via costimulatory molecules, prime the immune system by cytokine and chemokine secretion and maintain CD8 T cells responses (7). Moreover, CD4 T cells have direct effector activity, including performing cytotoxic functions (8) and mediating macrophage activation. Dysregulation of CD4 T cells is associated with many chronic diseases. CD4 T cells according to their phenotype and function are categorized into different subsets. Th1, Th2, Th17 and Regulatory T cells are the most well-known T cell subsets (Figure 1). Polarizing cytokines, master gene regulators and cytokine signature are three major characteristics of each subset.

Th1 subset

Interleukin-12 (IL-12), IL-18 and Interferon γ (IFNγ) are key cytokines in Th1 polarization. IL-12 and IL-18 are cytokines that are produced by macrophages and DCs and which, together with IFNγ produced by T cells, induce Signal Transducers and Activators of

Transcription 4 (STAT4) (9) and up-regulate expression of T box expressed in T cells (T-bet) as master regulator of Th1 cells. T-bet expression promotes Th1 commitment and induces Th1 cells to produce IFNγ (10). IFNγ, TNF and lymphotoxin α (LTα) are the most important Th1 cytokines (11). Th1 cells are involved in macrophage activation, antibody class switching (in B cells) and promotion of fully cytotoxic T cell activity. These functions by Th1 cells contribute to responses against viral and intracellular pathogens.

Th2 subset

IL-4 is the critical cytokine for polarization of Th2 cells and their differentiation is controlled by the master transcription factor GATA3. IL-4 acts by signaling via STAT6, which is the necessary signal transducer for Th2 differentiation (12, 13). IL-4, IL-5 and IL-13 are signature cytokines of Th2 cells (14). The main role of Th2 cells is clearance of extracellular parasitic infections. The secreted cytokines by Th2 cells induce eosinophil differentiation and promote production of IgE and IgG1 by B cells. Th2 cells also have important roles in mediating allergic reactions (15).

Th17 subset

The characterization of IL-17 producing T cells as a distinct T cell lineage was based on studies performed in the mouse model of MS, experimental autoimmune encephalomyelitis (EAE) (16, 17). Differentiation of Th17 cells from naive CD4 T cells is controlled by RORc as master regulator (18). TGFβ (immunoregulatory cytokine) and IL-6 (proinflammatory cytokine) are two main cytokines which are required to induce IL-17 in naive T cells. Rorc expression is inhibited by a high concentration of TGFβ, while presence of TGFβ in combination with IL-6 causes induction of IL-17 expression (19, 20). IL-23 is another important cytokine in Th17 differentiation, and it has been considered as a cytokine needed in order to up-regulate IL-22 by Th17 cells (21) and necessary to attain full pathogenic potential through its maintenance and stabilization of the Th17 phenotype in autoimmunity (22). STAT3 is the main signal transducer in Th17 cells and not only causes increased expression of RORc molecule but it can directly bind to IL-17 and IL-21 promoters (23, 24). The main function of IL-17 in immunity against pathogens is due to its induction of pro-inflammatory cytokines and chemokines which orchestrate trafficking and activation of innate immune cells particularly neutrophils and macrophages (25). Many inflammatory or autoimmune diseases which were previously considered to be Th1-mediated disorders are now considered as Th-17-mediated diseases.

IL-17 is actually a family of cytokines, which includes IL-17A (called IL-17), IL-17B, IL-17C, IL-17D, IL-17E (called IL-25), and IL-17F (19). Among the IL-17 family members, IL-17A and IL-17F have the highest degree of homology and both IL-17A and IL-17F are produced by a variety of cell types and have pro-inflammatory properties (26). IL-17 family receptors also consist of five members (IL-17RA, RB, RC, RD and RE), all of which, like their ligands, share sequence homology (27). IL-17A and IL-17F signal through the IL-17RA–IL-17RC heterodimeric receptor complex (28).

Regulatory T cell (Treg) subset

Regulatory T (Treg) cells is a distinct T cell subpopulation that is engaged in sustaining immunological self-tolerance and homeostasis (29, 30). The X-chromosome-encoded member of the Forkhead Transcription Factor box P3, named Foxp3 is a master regulator of

regulatory T cells (Treg) which controls differentiation and function of Tregs (31). Currently CD25 (IL-2 receptor subunit α) is the best surface marker to define Treg cells, although several markers have been described for Treg identification like CTLA-4 (cytotoxic T lymphocyte antigen-4), GITR (glucocorticoid-induced TNF receptor family), CD127 ^{low} and IL-1 receptor although they are not completely Treg specific (32-35).

The majority of Tregs develop in the thymus by high-avidity recognition of self-antigens and act to prevent damage caused by self-reactive T cells which evade the negative selection process (T cells with high affinity for self MHC/peptide); this group of T cells is named thymus-derived Tregs (tTreg) or natural Tregs (nTreg) (36). Another group of Tregs named peripheral Treg (pTreg) or induced Treg (iTreg) develop in the periphery in response toT cell receptor (TCR) stimulation in combination with several other signals, including IL-2 and TGF-β (31, 37). Foxp3+ Tregs, by migration to inflammatory sites are responsible for the suppression of various effector cells.

There is no well-accepted marker to distinguish tTregs from pTregs. Helios is a member of the Ikaros transcription factor family that has been considered as a marker of natural or thymic-derived Treg cells recently (38), although this notion has been challenged later on (39) and more recently additional roles for Helios has been suggested like a mediator of T-cell homeostasis or a T-cell activation and proliferation marker (40, 41).

Tregs exert their suppressive function by several modes of action that can be classified into five main mechanisms. Induction of suppressor cells or 'infectious tolerance' is mediated by TGFβ by induction of IL-10 expressing Tr1-like cells. IL-10, IL-35 and TGFβ are cytokines that are released by Tregs and have suppressive function. Tregs also can modulate APC cell function; for instance CTLA4 is a negative costimulatory factor that binds to CD80/CD86 (on APC) and mediates induction of indoleamine 2,3-dioxygenase (IDO), which is an immunosuppressive molecule made by DCs. Tregs can also mediate cytolysis by granzyme A, granzymeB and perforin. Metabolic disruption is the last proposed mechanism in order to induce suppressive function. CD25 (part of the IL-2 receptor) is a surface marker on Tregs that can deprive effector T cells of IL-2 (42, 43). Dysregulation in the function of Tregs contributes to the pathogenesis of many chronic inflammatory diseases and autoimmune diseases (44, 45).

Tr1 and Th3 are other regulatory T cells with no Foxp3 expression. They have suppressive activity on effector cells by IL-10 and TGFβ production respectively (42).

Other subsets of T cells

In recent years the number of effector T helper subsets has grown further. Follicular helper T cells (Tfh), Th9 and Th22 are defined as distinctive T cell subsets but much still remains unknown about them.

Recent studies suggest follicular helper T cells (Tfh) are a distinct lineage of CD4 T cells which act in the germinal center of peripheral lymphoid tissue where they provide help to maintain and to regulate germinal center B cell differentiation into plasma cells and memory B cells (46-48). Differentiation of Tfh cells is under control of Bcl6 as a master regulator and dependent on IL-21, IL-6 and STAT3. Human Tfh cells have been implicated in various diseases like immunodeficiency and autoimmunity (49).

Th9 is another newly described subset of T cells with preferentially production of IL-9. They develop from naive T cells in the presence of TGFβ and IL-4 (50, 51). GATA3 and STAT6 are transcription factors and signal inducers that are required for Th9 differentiation. Involvement of Th9 cells in the pathophysiology of allergic asthma and autoimmunity has been demonstrated (52).

Th22 is a new subset of T cells that by a unique gene expression pattern and function make it distinct from Th17 cells. The aryl hydrocarbon receptor (AHR) is an important transcription factor in Th22 cells and IL-6 and TNF are cytokines that promote Th22 differentiation. Th22 cells, depending on the precise cytokine milieu and affected organ may modulate their functional profile showing both protective or pathogenic characteristics (53-55), and ongoing research aims to understand their complicated role in inflammatory and autoimmune diseases.

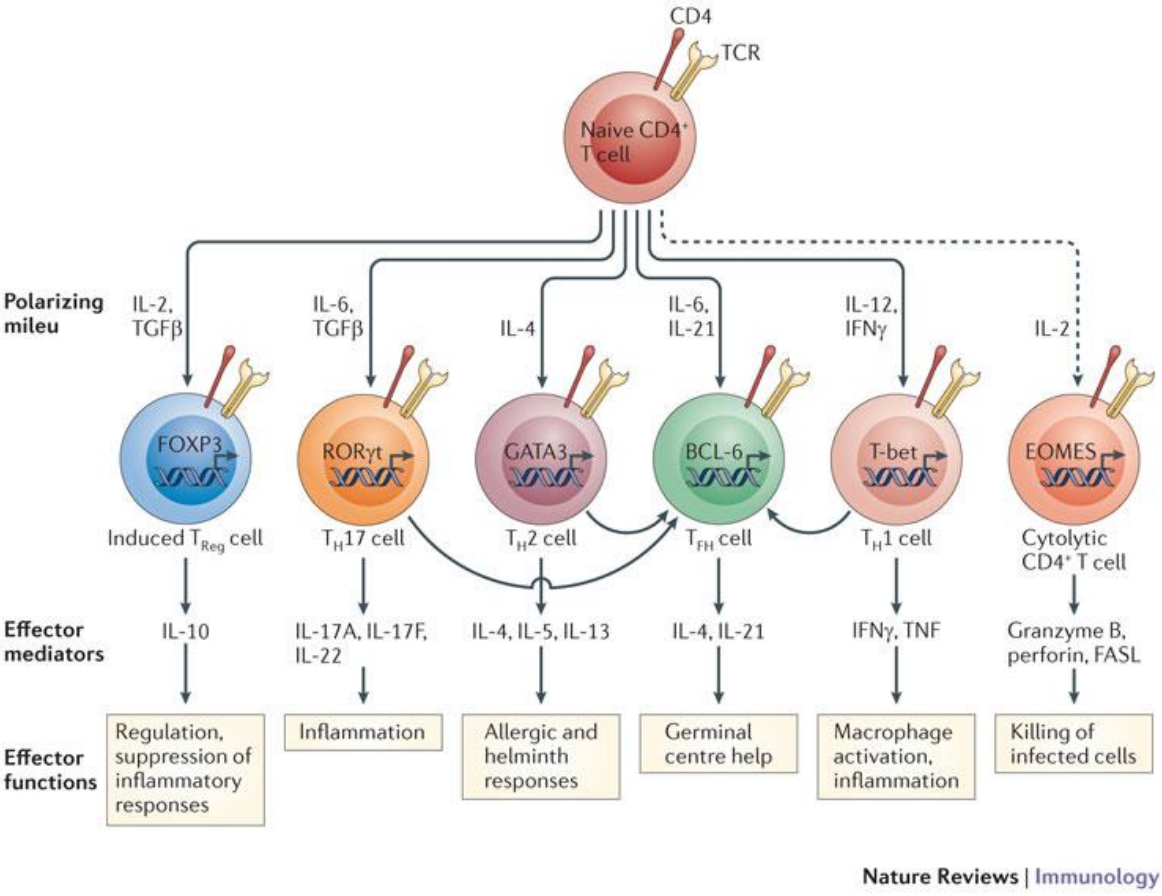

Figure 1: T cell subsets differentiation. Following recognition of cognate antigen that is presented by APCs, naive $CD4^+$ T cells become polarized into distinct effector T helper cell subsets in lymphoid organs. The cytokine milieu plays an important role in orchestrating T cell polarization. (adapted from Nature Review Immunology 12:136-148) (56).

T cell plasticity

T cell plasticity is a phenomenon that has been recently described to occur in T cell subsets. Plasticity is defined as an ability of a T cell subset to display a flexible cytokine production pattern that includes cytokines normally considered to strictly belong to different T cell lineages. The most well-known example of a flexible T cell lineage was described in the case of Treg versus Th17 maturation, where TGFβ mediates differentiation of Foxp3+ Tregs from naive T cells in response to antigenic stimulation while the presence of TGFβ together with IL-6 leads to differentiation of Th17 cells from naive T cells (57). Among the T cell subsets, Th1 and Th2 cells cross-regulate each other by inhibiting the development of the other T cell subset, but following differentiation they have a stable phenotype and have achieved a lineage commitment state (58). In contrast, there is strong evidence indicating that a fraction of Foxp3+ Treg cells can acquire effector T cell function by losing Foxp3 expression under certain circumstances. The frequency of these ex-Foxp3+ Tregs increases particularly in the presence of inflammation (59). However, under inflammatory conditions a fraction of Foxp3+ Tregs can also acquire certain effector T cell functions such as IFNγ production with retained Foxp3 expression (60). These regulatory T cells have a high level of Foxp3, lack of IL-2 production and a maintained suppressive activity (61). Transition of Th17 cells that predominantly produce IL-17 to IFNγ producing cells has also been reported by different groups (62). The exact function of these flexible T cell subsets remains to elucidate but they may contribute to the protection against microbes, development of autoimmunity and anti-tumor activity (63). Regarding Th9 cells with IL-9 production it is not clear whether they should be considered a distinct T cell lineage or if they reflect an adaptation of Th2 cells under certain circumstances. Flexibility and similarity between Tfh and both Th1 and Th2 cells also makes it unclear whether Tfh is a separate T cell lineage or rather a 'chameleon' state of other subsets (64).

1.4.2.2 CD8+ T cells

CD8 T lymphocytes, named cytotoxic T lymphocytes (CTL), exert their main effect by killing target cells, such as virus-infected or tumour-transformed cells. CD8 T cells like CD4 T cells have T cell receptors on their surface and they recognize peptide antigens which are presented by MHC class I molecules on the surface of infected cells. Following recognition of antigen by their TCR they are activated and they kill the target cells by delivery of granule proteins. Granzymes and perforins are two major components that CTLs release to kill target cells. Granzymes are enzymes that induce apoptosis in target cells and perforin is a delivery protein that mediates transfer of granzymes. CD8 T cells also carry Fas ligand on their membrane that can induce cytotoxicity by binding to Fas (CD95, a TNF receptor family member) which delivers a death signal if binds to its ligand). Activated CTLs also have the ability to secrete many cytokines such IFNγ, TNF and IL-17 which are important in immune activation. CTL are critical T cells for eradication of intracellular pathogens like viruses (3).

1.5 Antigen presentation and human leukocyte antigen (HLA)

T cells can recognize cognate antigens by their surface T cell receptors, if antigens have been presented to them by major histocompatibility complex (MHC) molecules. Since the MHC molecules first were discovered on the surface of human leukocytes, they are also named human leukocyte antigen (HLA) complex. The MHC encoded genes in human are located on chromosome 6 as part of a locus which contains many genes which can classified in three

groups, named MHC class I, MHC class II and MHC class III. MHC class I and II are involved in presenting peptide antigens to T cells, while MHC class III contains genes for some cytokines and complement proteins and does not have any role in antigen presentation.

Despite high similarity in the structure and function of MHC class I and II they also differ in many characteristics, and some of the major differences are summarized in table 1 (65, 66).

	MHC class I	MHC class II
Source of antigen	Endogenous antigens	Exogenous antigens
Responding cells	$CD8^+$ T cells	$CD4^+$ T cells
Composition of molecule	α1, α2, α3/ β2 macroglobulin	α1, α2/ β1, β2
Encoded genes	α chain encoded by MHC gene and β chain by non MHC genes	Both α and β chain encoded by MHC gene
Structure of molecule on cell surface	Only α chain span the cell membrane	Both α and β chain span the cell membrane
Presented cells	Expressed by most nucleated cells	Expressed by APCs (Macrophage, DC and B cell)
Peptide binding groove	Both ends close	Both ends open
Size of presented peptide	8-10 amino acids	13-18 amino acids

Table 1: Different characteristics of MHC class I and MHC class II molecules

MHC class I encodes in humans the three genes HLA-A, -B and -C, while MHC class II encodes HLA-DP, -DQ and -DR. MHC allelic genes inherited from both parents are expressed equally. The gene region of MHC molecules is highly polymorphic and contains multiple gene variants with the same function and slightly different structure, for instance HLA-DRB1genes are encoded by 860 different allotypes (some are very common while others are very rare). This polymorphism leads to diversity in the expressed MHC repertoire and it is extremely rare to find two individuals with the same set of MHC molecules in the population (except for siblings) (1).

1.6 The T cell receptor

As described above, APCs present antigens via MHC molecules to the T cells. In order to induce appropriate immune responses against pathogens, the antigen should be recognized by specific cognate antigen receptors on the surface of T cells. T cell receptors like B cell receptors consist of a constant plus a variable domain, where the latter is antigen-specific and the enormous diversity of the total TCR repertoire enables T cells to recognize a wide variety of peptides.

TheT cell receptor consists of an α polypeptide chain and a β polypeptide chain and each of them is composed of a variable and a constant region. The variable region of the TCR α chain

is encoded by V (variable) and J (junction) gene segments and the variable region of the β chain is encoded by V, D (diversity) and J gene segments. Each of these V, D and J segments can be selected from a number of alternative gene segments that after somatic gene rearrangement are combined to generate the complete variable α and β chains. This rearrangement creates vast diversity in variable regions of T cell receptors by the many different possible combinations of gene segments.

The three dimensional structure of a T cell receptor in the antigen binding sites reveals hypervariable loops, named complementarity determining regions (CDR). T cell receptors have three different CDR loops on each chain, among them CDR3 has the most variable loop and make the center of the antigen binding site of a T cell receptor. It is formed by contributions of D and J gene segments of the α and β chains. A major part of TCR diversity is generated by random inclusion/deletion of nucleotides at the junctions between V, D and J segments (1, 3).

A minority of T cells, termed γδ T cells, carry a different type of T cell receptor which instead of α and β chains is composed of γ and δ chains. These cells have completely different features compared to the αβ T cells and γδ T cells are part of the innate immunity system.

1.7 T cell activation

T cell maturation occurs in the thymus. During this process naive T cells expressing T cell receptors with capacity to recognize pathogenic antigens presented by MHC molecules develop following positive selection (to select T cells that recognize self MHC molecules by their T cell receptor) and negative selection (to eliminate T cells that bind to MHC with high affinity, a major mechanism to prevent autoimmunity) . Naive T cells leave the thymus and circulate between lymphoid organs to meet the cognate antigen presented by APCs. In order for activation of T cells to occur, antigen recognition by the T cell receptor is essential but it is not sufficient. In addition to T cell receptors a complex of co-receptors and co-stimulatory factors are necessary for proper T cell activation (67).

The CD3 molecule (T cell specific marker) and ζ-chain are co-receptors that generate an activation signal upon peptide-MHC recognition by T cell receptors. T cell receptor, CD3 and ζ-chain all together make up the T cell receptor complex. CD4 and CD8 are two other co-receptors expressed on the surface of T helper and cytotoxic T cells, respectively. When T cell receptors recognize peptide and MHC complex, CD4 binds to MHC class II and CD8 binds to MHC class I, ensuring that, depending on the antigen source, the correct type of effector cell is engaged.

There are a couple of co-stimulatory receptors that are essential for T cell activation. CD28 is a co-stimulatory receptor that mediates initiating of the activation signal in naive T cells. CD28 binds to B7 (CD80 and CD86) molecules which are expressed on APCs surface (68, 69). A group of integrin molecules are expressed on T cells, whose ligands are found on APCs. Leukocyte function-associated antigen-1 (LFA-1) is one of the important integrins which is involved in T cell activation and its ligand on APCs is intercellular adhesion molecule-1 (ICAM-1) (70). CD40 ligand (CD40L) on T cells is another molecule which indirectly participates in T cell activation. CD40L binds CD40 on the APC surface, inducing APCs to secrete cytokines like IL-12 (a cytokine that is necessary for Th1 cell differentiation) and enhancing expression of B7 molecules (71). There is also a set of negative co-stimulatory

molecules like CTLA-4 and PD-1 which promotes negative signals and inhibit T cell receptor signaling (72). CTLA-4 is a receptor that is expressed on the T cell surface and can bind to a B7 molecule instead of CD28 (73) (Figure 2).

After antigen recognition by the T cell receptor and provided that necessary co-stimulatory signals are present, T cells initiate a process involving activation of biochemical signaling pathways and expression of a variety of proteins and transcription factors that leads to proliferation and clonal expansion, differentiation and obtaining full effector function. Activated effector T cells leave the peripheral lymph nodes and migrate to the site of infection or inflammation (74).

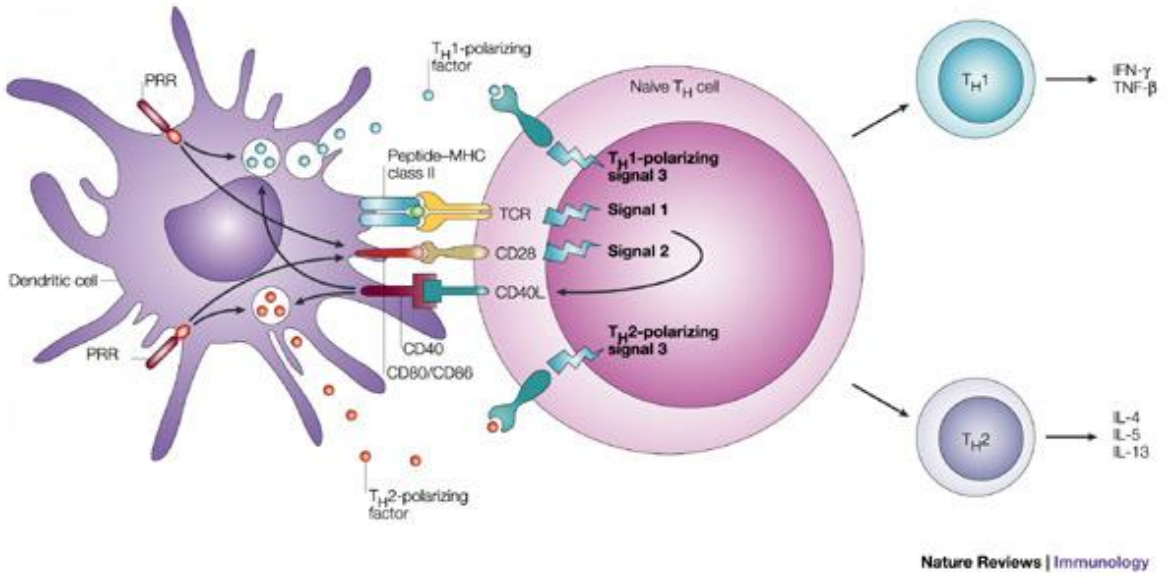

Figure 2: T cell activation initiated by three signals. Signal 1 is mediated by T cell receptors that recognize antigens presented by MHC molecule. Signal 2 is the co-stimulatory signal, mainly mediated by triggering of CD28 by CD80 and CD86. Signal 3 is the polarizing signal that mainly is provided by various soluble or membrane-bound factors, such as interleukin-12 (IL-12) that promote the development of Th1 cells (adapted from Nature Review Immunology 3: 984-993) (75).

1.8 The respiratory system

The lung is the main organ of the respiratory system whose responsibility is to provide the body with oxygen and remove carbon dioxide. The respiratory system is divided into two major parts: the upper and lower parts. The upper respiratory tract (upper airway) consists of the nose, nasal cavity, mouth, pharynx, larynx. The responsibility of upper respiratory system is to provide proper air conditioning (humidity and temperature), particle removal and air filtration and also to activate the immune defense against pathogens.

The lower respiratory tract consists of the trachea, bronchi, bronchioles and alveoli. The trachea enters the thoracic cavity and carries air from the throat into the lungs and vice versa. The trachea branches into bronchi and these are in each lobe of the lung further divided into smaller branches of airways called the bronchioles. Finally the bronchioles end into air sacs termed the alveoli. The alveoli have very thin walls surrounded by capillaries which permit exchange of gases with blood via passive diffusion; i.e. the main function of the respiratory system (Figure 3).

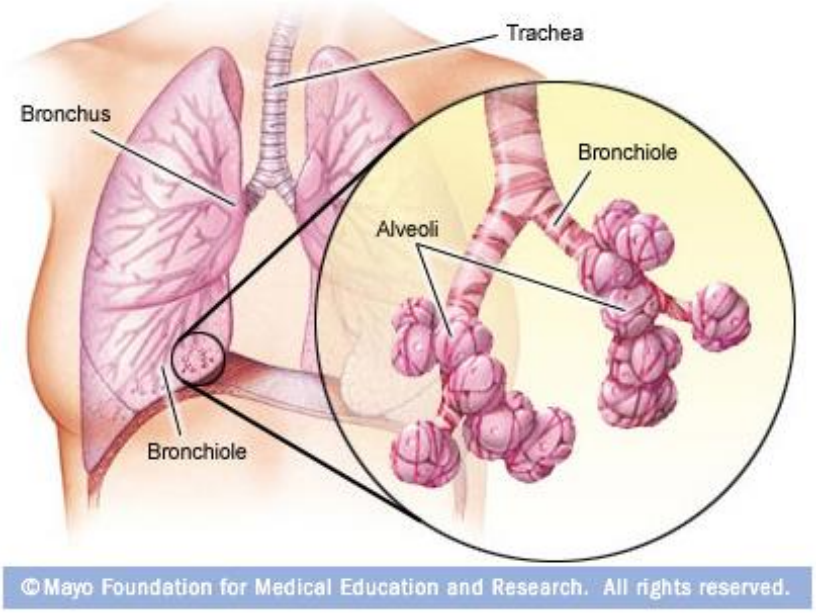

Figure 3: The major features of the lower respiratory system including the trachea, bronchi, the bronchioles, and the alveoli.

1.8.1 Immunology of the lungs

The lungs have the largest epithelial surface of the body and according to their function they are constantly exposed to microorganisms and environmental irritants. The lung epithelium is an important physical barrier, which is the first line of defense and acts as a shield to protect the body against irritants and pathogens including airborne toxins, cigarette smoke, pollutants, bacteria and viruses. However, the lung epithelium is not only a physical barrier but also it is activated in response to pathogen exposure or environment-induced damage to secretion of antimicrobial peptides and inflammatory mediators to recruit immune cells to the site of infection or damage (76).

The epithelium is composed of different cell types with a variety of functions which help the lung to protect itself against airborne insults. More than 50% of lung epithelial cells are named ciliated cells that by ciliary motility help mucous clearance (77). Goblet cells are another kind of epithelial cells that secrete mucous into the airway to trap pathogens and dust particles (78). The mucous layer in the airway is found from the trachea to the bronchioles

and it consists of a mixture of highly glycosylated mucin proteins that help to maintain lung health (79). In the smaller airways the secretory cells are replaced by Clara cells which are non-ciliated bronchiolar secretory cells. Clara cells have been shown to produce bronchiolar surfactants and specific enzymes to protect and support lung function (80, 81). Epithelial cells in alveolar walls and the ciliated epithelium also have pattern recognition receptors (PRRs) which can sense pathogens (82).

In addition to these physiological barriers, the lung is recognized as a unique immunologic organ equipped to protect the body from inhaled invading pathogens. If the lung with specific physical and physiological clearance properties can't control or remove inhaled particles, they can trigger the immune system in order to process foreign antigens. A variety of immune cells such as alveolar macrophages, lymphocytes and neutrophils are involved in lung protection. Alveolar macrophages account for approximately 95% of airspace leukocytes, lymphocytes with 1 to 4% and neutrophils only about 1% in the normal situation (82).

The alveolar macrophage (AM) is the resident mononuclear phagocyte in the alveolar air spaces. AMs can directly phagocyte inhaled harmful particles, moreover they are not only a potent orchestrator in the presentation of antigen to the T cells, but also they release different mediators to activate and recruit other immune cells (83). The AM can also produce reactive oxygen and nitrogen species and proteolytic enzymes to kill the ingested pathogens (84). Neutrophils also have an important role in lung immunity to pathogens. Neutrophil infiltration should be under control and in chronic airway diseases persistence of neutrophils in the lungs may lead to tissue damage due to their cytotoxic function (85, 86). Different types of lymphocytes are found in the lungs of healthy individuals such as T cells, B cells, NK cells, γδ T cells. Dendritic cells are professional APCs which are present just below the airway epithelium and by migration from the airway mucosa to the thoracic lymph nodes play an important role in the activation of T cells (87).

Insufficient or exaggerated immune responses to inhaled stimuli could lead to pathological conditions, such as pneumonia, asthma, chronic obstructive pulmonary disease (COPD) and interstitial lung disorders. Pulmonary inflammation affecting the airways or interstitium can be caused by many reasons and result in different manifestations. Both innate and adaptive immune systems have pivotal roles in orchestrating the inflammatory reaction and one of the most important cells in this process is T helper lymphocytes. Pulmonary inflammation is defined as increased inflammatory cells in the airspaces and lung tissue that are producing proinflammatory mediators such as hydrogen peroxide, interleukin-1 (IL-1), and interleukin-8 (IL-8) (88). The pulmonary inflammation can lead to obstructive symptoms like in asthma and COPD where inflammation causes narrowing of the airways or it can result in a restrictive pattern like in interstitial lung disease where inflammation leads to increased lung stiffness.

1.8.2 Interstitial lung diseases

The interstitial lung diseases (ILDs), also called diffuse parenchymal lung diseases, are a diverse group of pulmonary disorders classified together because of similar clinical, physiologic, or pathologic features (89, 90). They can be caused by a variety of factors and are clinically characterized by diffuse infiltrates on the chest radiograph and histologically by distortion of the gas exchanging portion of the lung. The physiologic correlates are restriction of lung volumes and impaired oxygenation (91, 92). Idiopathic pulmonary fibrosis (IPF) (a

progressive and lethal fibrotic lung disease), nonspecific interstitial pneumonia, chronic beryllium disease, silicosis and sarcoidosis are examples of ILDs.

1.8.2.1 Sarcoidosis

Sarcoidosis is a systemic inflammatory disorder. It is characterized by non-caseating granulomas that most commonly affect the lungs. The pathogenesis of sarcoidosis includes the accumulation of lymphocytes and macrophages in the alveoli; thus involving innate as well as adaptive immune mechanisms. The prevalence of sarcoidosis is about 4.7-64 in 100,000 and its incidence is about 1-35.5 in 100,000 per year. The northern European and African-American individuals have the highest rate of sarcoidosis (93, 94).

Clinical aspects

The main clinical symptoms in pulmonary sarcoidosis patients are non-productive cough and dyspnea, although other symptoms like fatigue (associated with impaired quality of life) and pain (especially arthralgia) are common in patients with pulmonary sarcoidosis (95, 96). Sarcoidosis can affect many different organs such as liver, spleen, lymph nodes, salivary glands, heart, nervous system; however the lung is the most commonly affected organ (97).

In general, a radiographic staging system is used in order to classify patients with pulmonary sarcoidosis. In this system a normal chest radiograph is classified as stage 0, stage I shows bilateral hilar lymphadenopathy (BHL) without pulmonary infiltrates, stage II BHL with pulmonary infiltrates, stage III pulmonary infiltrates without BHL and stage IV fibrosis with distortions. The extent of pulmonary engagement, reflected through the chest X-ray classification, is associated with the prognosis (this is the case in 90% of patients) (97).

Sarcoidosis patients generally have a rather good prognosis and nearly two-thirds of the patients recover spontaneously, however a chronic progressive disease course is seen in 10%–30% of patients (98, 99). In late course of chronic disease, pulmonary fibrosis may occur in 20 to 25% of patients and the rate of mortality is 1%-5% among sarcoidosis patients(97, 100).

Pathogenesis

The aetiology of sarcoidosis is still not known. However, associations with environmental factors, genetic susceptibility and bacterial triggers have been reported (101, 102). Due to involvement of lungs, eyes and skin, environmental causes have been considered for many years. An association of sarcoidosis with exposure to irritants such as tree pollen, inorganic particles, and moldy environment has been reported (103-105). Occupational studies have shown an increased risk for sarcoidosis in people who work in U.S. navy, firefighting and metalworking (100, 106, 107).

The presence of mycobacterial and propionic bacterial DNA in the lungs of sarcoidosis patients has been shown (108, 109). Accumulating data suggest that mycobacteria and in particularly *M. Tuberculosis* play a major etiologic role in sarcoidosis (110). It is not clear how infectious agents cause sarcoidosis, although one hypothesis is that sarcoidosis is caused by a viable, replicating mycobacterial or other infection with no microbiologic, pathologic and clinical evidence (111). However, most investigators favor a view of sarcoidosis as caused by remnants of an infectious agent in the form of poorly degradable antigens. The involvement of *M. Tuberculosis* in the pathogenesis of sarcoidosis has been validated by

immune assay studies (112). Ex vivo immune activation in response to several mycobacterial antigens have been studied and T cell responses to the mycobacterial virulence factor antigen 85A (Ag85A), early secreted antigenic target protein-6 (ESAT-6), culture filtrate protein-10 (CFP-10) and heat-shock proteins (hsp) have been reported in sarcoidosis patients (113-115).

mKatG

Homogenates of spleen and lymph node from sarcoidosis patients were previously used in the diagnosis of sarcoidosis. The test is named Kveim´s test and it is performed by intradermal injection of tissue homogenate that results, in sarcoidosis patients, in formation of granulomas at the site of injection (116). A proteomic approach was undertaken to identify antigens in sarcoidosis patients´ tissue samples, treated in the same way that Kveim´s reagent is prepared. *Mycobacterium tuberculosis* catalase-peroxidase (mKatG) was one of the non-self tissue antigens identified and antibodies to recombinant mKatG were detected in the sera of 12 of 25 (48%) sarcoidosis patients (117). This finding indicated that mycobacterial antigen is present, at least in a subset of sarcoidosis patients (112). T cell responses against mKatG have been observed in sarcoidosis lung and peripheral blood mononuclear cells (118, 119), and a study on lung mKatG-reactive, IFNγ-producing effector T cells indicated that T cell responses to mKatG in sarcoidosis are regulated in a manner expected for a pathogenic antigen (120).

Immunopathogenesis

Non-necrotizing granulomas are the histological hallmark of sarcoidosis. The granuloma formation occurs in the body whenever an antigen cannot be completely eliminated or degraded by macrophages. In this situation macrophages and epitheloid cells fuse together and make multinucleated giant cells; moreover accumulation of activated mononuclear cells (mostly T cells) and interaction between innate and adaptive immune cells by release of proinflammatory cytokines orchestrates the formation of granulomas and induce an immune stimulation in the affected organ (121-123) (Figure 4).

In bronchoalveolar fluid of sarcoidosis patients the proportion of T cells is increased (20–60% of the total cell count) with dominance of CD4+ T cells. An increased CD4/CD8 ratio in bronchoalveolar fluid of patients (>3.5) is one of that characteristics of sarcoidosis patients (124). The accumulated activated CD4+T cells in alveoli are typically Th1 cells and they are considered to be of central importance for the inflammatory process, although CD4+ T cells in lung tissue and bronchoalveolar cells bearing a phenotype consistent with Th17 polarization and persistence of Th17 cells in sarcoidosis patients has been observed (125-127). By contrast, Th2 cell responses (IL-4 and IL-5 secretion) are downregulated at sites of inflammation in sarcoidosis patients (128, 129).

T cells in BAL fluid of sarcoidosis patients are highly activated and they express activation markers like CD69 and HLA-DR. A Th1 cytokine signature i.e. IFNγ is highly expressed, moreover cytokines that promote a Th1 response such as IL-12, IL-18 and IL-27 are upregulated in the lung of sarcoidosis patients (127, 130, 131). The expression of Th1-associated chemokine receptors such as CXCR3 and CCR5 has also been reported in lung of sarcoidosis patients (132).

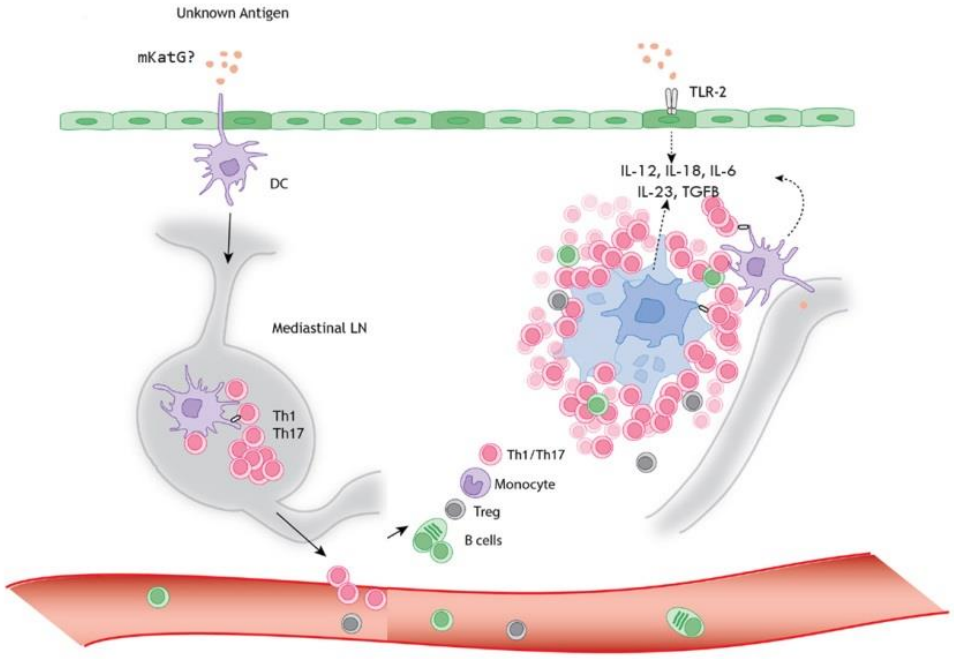

Figure 4: Schematic speculation process of granuloma formation in sarcoidosis patients (Adapted from (133) with minor modification).

Treatment

There is no cure for sarcoidosis but conventional treatment improves the clinical consequences of disease by alteration of the granulomatous process (134). The decision of which treatment to use is dependent on clinical characteristics and severity of disease (135). Spontaneous resolution has been observed in many patients and treatment can usually be avoided, but oral corticosteroids are usually prescribed in patients with pulmonary sarcoidosis who suffer from lung dysfunction and persistent pulmonary infiltrates. The first line of treatment in severe sarcoidosis is corticosteroids (98, 136). There are other strategies in treatment of sarcoidosis patients such as cyclosporin to inhibit activated T cells or TNF blockade given the importance of TNF in the initiation and perpetuation of the granulomatous process, although there is no consensus regarding the efficiency of these treatments and their effect varies in different patients (137-139).

Löfgren's syndrome

Sarcoidosis patients display phenotypic heterogeneity. A subgroup of sarcoidosis patients with specific clinical features, described the first time by the Swedish pulmonary physician Sven Löfgren (1910-1978), are said to have Löfgren's syndrome. Löfgren's syndrome (LS) is an acute form of sarcoidosis with bilateral ankle arthritis and/or erythema nodosum, usually fever, and bilateral hilar lymphadenopathy (140, 141).Löfgren´s syndrome has a good prognosis in general and spontaneous recovery within two years is particularly common in

Löfgren´s syndrome patients who also express the HLA allele DRB1*0301 (DR3) (142). HLA-DR3+ patients (two thirds of which have LS) virtually always have accumulations of TCR Vα2.3+ CD4+ T cells, i.e. T cells with a particular T cell receptor (TCR) alpha chain variable gene segment (AV2S3) (TCR-AV2S3) in the lungs (143). Such expansions were not found in DR3+ healthy individuals or in patients with other inflammatory pulmonary disorders (144, 145). More recently, these Vα2.3+ T cells in DR3+ patients were found to often use Vβ22 as part of their TCR β chain (146)(Kaiser *et al*, unpublished observations). The reasons for spontaneous recovery in some patients and disease progression in others remain to be elucidated. However, hypothetically in non-necrotising granuloma remnants of an assumptive antigen are not fully degraded and antigen persistence leads to progressive chronic disease (122, 147). In contrast, it can be hypothesized that the immune responses in patients with spontaneous resolution results in complete elimination of causative antigen (134).

1.8.3 Smoking induced-inflammation

Cigarette smoking by more than one billion consumers in the whole world is one of the most challenging health issues due to its effect on lung and other organ systems resulting in a high morbidity and mortality (148). Cigarette smoke consists of more than 6000 different chemicals and toxic components, such as nicotine, aromatic hydrocarbons, dioxin, tobacco glycoprotein, phenol, metals and ions which some of them are cytotoxic, antigenic and carcinogenic (149-151). Tobacco smoking is a well-known key factor in the pathogenesis of several diseases, such as infection, lung cancer, cardiovascular diseases, COPD and autoimmune disorders.

Cigarette smoke influences the airways by directly inducing activation of epithelial and immune cells. Cigarette smoke influences both innate and adaptive immunity at the local or systemic levels in different ways (151). The chronic exposure to components of cigarette smoke triggers a variety of pulmonary or systemic immune responses. Smoking can induce or inhibit release of both inflammatory and anti-inflammatory cytokines and mediators (151). Cigarette smoke through up-regulation of NF-κB (152) can induce secretion of inflammatory cytokines such as TNF, IL-1, IL-6, IL-8 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (153, 154). Induction of pro-inflammatory mediators leads to recruitment of immune cells and consequently inflammation. However, cigarette smoke can also down-regulate the release of IL-1β, IL-2, IFNγ and TNF via toll like receptors (155). Nicotine is one of the components of cigarette smoke that show direct anti-inflammatory effect by diminishing IL-6 production (156). Furthermore nicotine can activate α7 nicotinic acetylcholine receptor that leads to down-regulation of pro-inflammatory cytokines like TNF, IL-1b and IL-6 (157). Cigarette smoke by different mechanisms modulates epithelial and immune cell signaling to induce suppression of aspects of innate and adaptive immune cell activation which hinder immunity to infection (158, 159).

T helper lymphocytes at the center of adaptive immunity have a pivotal role in orchestrating the host defense and inflammatory reaction following triggering by specific antigens. Smoking has an influence on different T cell subsets with specific inflammatory or anti-inflammatory cytokine properties. Imbalance between pro-inflammatory and anti-inflammatory cytokines in the lungs can result in pathologic inflammatory conditions.

Smoking-induced autoimmunity

Cigarette smoking has been shown to be associations with autoimmunity. There is strong evidence of an increase in prevalence of many autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematous, Crohn's disease and systemic sclerosis in smokers (151, 160, 161).

Rheumatoid arthritis (RA) is an autoimmune disorder and a striking example of gene-environment interaction as the combination of smoking history and having two copies of HLA-DR "shared epitope" genes increased 21-fold the risk of the RA form characterized by antibodies to citrullinated protein antigen (ACPA) (162). Part of the mechanism appears to be that cigarette smoke induces the PAD enzymes responsible for citrullination (a post-translational modification) of proteins such as vimentin (163). Further examples of autoimmune disorders where cigarette smoking plays a role are systemic lupus erythematosus (SLE), where a smoking history was associated with more active disease and increased levels of anti-dsDNA antibodies (164).

Smoking-induced multiple sclerosis

Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), characterized by multifocal demyelination of nerves with relative preservation of axons and accompanied by oligodendrocyte loss (165-168). T-cells have a central role in the pathophysiology of MS and presence of T cells in CNS lesions and infiltrates of T-cells throughout the CNS is a hallmark characteristic in all stages of MS patients (169). Activated T-cells occur at a higher frequency in peripheral blood of MS patients (170).

The risk of developing multiple sclerosis (MS) is strongly influenced by smoking in individuals with genetic susceptibility (certain HLA alleles). An interaction between the presence of HLA-DRB1*15 and absence of HLA-A*02 among smokers leads to a 17-fold increased risk of MS compared with non-smokers without the genetic risk factors. Furthermore the risk increased further when the analysis was restricted to comparing current smokers with never-smokers (171). Smoking is associated with increased lesion volume and greater atrophy in MS patients (172).

Importantly, there is strong evidence in Lewis rat transfer experimental autoimmune encephalomyelitis (EAE) (a model of MS) that shows that the lung could contribute to the activation of potentially autoaggressive T cells and their transition to a migratory mode as a prerequisite to entering the central nervous system (CNS) (173).

2 AIMS

T cells of different subsets have a pivotal role in orchestrating the host immune protection and inflammatory reaction. Activated T cells in the lungs not only have the potential to affect that particular organ but due to lymphocyte trafficking they can also contribute to immune responses in other organs. The overall aim of the studies presented in this thesis was to investigate the characteristics of T cell immunity in chronic pulmonary inflammation in two different aspects:

A. Investigation of antigen-specific T cell responses in patients with pulmonary sarcoidosis. Project number 1 and 2 were done based of this objective.

- To elucidate the ability of T helper 1 cells in lung and blood of different sarcoidosis patient subgroups to respond with cytokine production to mycobacterial antigen (mKatG) stimulation,
- To study the single- or multifunctional cytokine profile of Th1 cells in response to mycobacterial antigen in the lung and blood of sarcoidosis patients.
- To evaluate the presence and levels of IL-17 and the frequency of IL-17 producing cells in response to mKatG and PPD in the lung and blood of patients with active sarcoidosis.
- To compare levels of IL-17 and the frequency of antigen specific IL-17 producing cells in lung and blood of different sarcoidosis patient subgroups.

B. Determining how smoking affects lung T-cell immunity, with regard to the influence of smoking in developing autoimmune disease

- To characterize of effector and regulatory T cell subsets in the lungs and blood of smoking and non-smoking healthy individuals.
- To elucidate the effector and regulatory T cell subsets in the lungs and blood of multiple sclerosis patients with regards to effect of smoking and treatment.

3 COMMENTS ON METHODOLOGY

The present thesis is based on studies of human samples from the airways and blood of sarcoidosis patients, MS patients and healthy individuals (smokers and non-smokers). The studies were performed after approval by the Regional Ethical Review Board (Stockholm, Sweden) and written consent was obtained from the all included subjects.

3.1 Study subjects

The sarcoidosis patients included in the present study were referred to the Lung and Allergy Clinic at the Karolinska University Hospital, Solna, Stockholm, Sweden for primary diagnostic investigation. The diagnosis was established using the criteria of the World Association of Sarcoidosis and other Granulomatous Disorders (WASOG) (97), based on chest radiographic picture, pulmonary function tests (vital capacity, forced vital capacity, forced expiratory volume in one second, and diffusing capacity of the lung for carbon monoxide), histology (positive biopsies), bronchoalveolar lavage (BAL) findings, and clinical symptoms compatible with sarcoidosis.

The clinical examinations of MS patients who participated in this study were performed by specialists in the Neurology clinic at the Karolinska University Hospital in Solna, Stockholm, Sweden and all patients diagnosed with MS fulfilled the McDonald criteria revised in 2010 (174). All MS patients were evaluated clinically at the time of sampling with the Expanded Disability Status Scale (EDSS). After diagnostic evaluation at the Neurology clinic, all patients were referred to the Lung and Allergy clinic for primary clinical examination with respect to the lungs and spirometry.

All healthy subjects in this study were examined at the Respiratory Medicine Unit (Karolinska University Hospital, Solna, Stockholm, Sweden). Included individuals had normal lung function and were in good health. They had normal chest X-rays and there were no signs of respiratory infection for at least one month prior to the bronchoscopy procedure.

DNA extraction was routinely done from whole blood of all subjects and PCR was run for analysis of HLA-DR by Olerup SSP™ PCR (Sequence Specific Primers for PCR).

3.2 Sampling

Bronchoalveolar lavage

Patients underwent bronchoscopy with bronchoalveolar lavage (BAL) in the morning. Morphine-scopolamine was injected intramuscularly, and topical anesthesia was sprayed into the nose and throat immediately before the BAL procedure (175). BAL was performed during flexible fiberoptic bronchoscopy after wedging the bronchoscope in a middle-lobe segmental bronchus. Sterile phosphate-buffered saline (PBS) solution was instilled in five aliquots of 50 mL. The solution was gently aspirated into a plastic bottle and kept on ice until use. In the laboratory, the BAL fluid was filtered to remove debris and mucus and the recovered volume was determined. BAL cells were washed two times with cold PBS and finally total cell number was determined by Bürker chamber counting.

The BAL cell differential count, including percentages of macrophages, lymphocytes, neutrophils, eosinophils, basophils and mast cells, was determined using May-Grünwald-Giemsa staining of cytospin slides.

Peripheral blood mononuclear cells

Whole blood was obtained in the morning of bronchoscopy and collected in heparinized tubes. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Hypaque density gradient separation (Pharmacia, Uppsala, Sweden). Equal volumes of blood and room-tempered (RT) phosphate buffered saline (PBS) solution (pH 7.4) were mixed and gently transferred to tubes containing Ficoll-Paque^{TM} Plus. The tubes were centrifuged (400g for 25 min at RT) and thereafter PBMCs formed a whitish layer just beneath the plasma. The PBMCs were carefully transferred into a new tube and washed 2 times with cold PBS. After last washing PBMCs were counted in a Bürker chamber in order to obtain the total cell number.

3.3 Methodology

T cell stimulation

T cell stimulation was done in order to evaluate T cell cytokine production. We have used different agents for T cell stimulation depending on the specific aims. In paper I, T cell stimulation was done by mKatG, purified protein derivative (PPD) from *Mycobacterium tuberculosis* and superantigen stimulation. The recombinant mKatG protein was isolated and prepared at the John Hopkins University School of Medicine, using an *Escherichia coli* strain (176). PPD is an extract of *Mycobacterium tuberculosis* PPD and it is widely used as a diagnostic antigen for tuberculosis by skin test. A total of 171 different proteins have been identified in the PPD mixture. PPD is commonly used for *in vitro* stimulation to characterize B and T cell reactions (177). We have also used a mixture of *Staphylococcus enterotoxin* A (SEA) and *Staphylococcus enterotoxin B* (SEB) as a positive control in our experiments (superantigens). Superantigens are bacterial or viral proteins with the ability to bind simultaneously to the α chain of HLA class II and a Vβ chain from the T cell receptor. Each superantigen has specificity for several of the Vβ versions. Superantigens are potent mitogens and powerful tools for T cell stimulation (178). A mixture of SEA and SEB thus in a physiological way has the potential to stimulate a large fraction of T cells.

During antigen stimulation, whole blood cells were further co-stimulated with anti-CD28 and anti-CD49d. Unstimulated BAL cells in medium alone and unstimulated whole blood were used as negative controls.

Brefeldin A (BFA) (GolgiPlug derived from *Penicillum*) is a protein transport inhibitor and was added to stimulated cells in order to block the intracellular protein transport process. It was added to the BAL and blood samples during the last 4 hours of *in vitro* antigen stimulation. Following T cell stimulation cytokine production was evaluated by flow cytometry.

In paper II, T cell stimulation was done in order to study mKatG specific Th1 and Th17 responses by Elispot. 10 μg/ml mKatG was used for T cell stimulation and anti-CD3 as a polyclonal stimulation was used as a positive control for stimulation of Th17 cells and Phytohemagglutinin (PHA) for Th1 stimulation.

In paper III and IV, intracellular cytokine and transcription factor staining was analyzed by flow cytometry. We stimulated both BAL and PBMC by anti-CD3 together with anti-CD28

in 37°C, humidified atmosphere of 5% CO_2 in air and unstimulated BAL and PBMC cells in medium alone were used as negative controls. Protein transport inhibitor (Brefeldin A) was added to the cells after 4 hours incubation followed by 12 hours incubation for a total of 16 h incubation. Stimulation in presence of Golgi Plug in cell culture for longer than 12 hours is lethal for the cells.

Flow cytometry

Flow cytometry was done on BAL cells, whole blood samples and PBMC for the study of phenotype and intracellular markers of T cells. A FACS Canto II (BD, Sweden) flow cytometer with filters to analyze eight colors was used.

The cell surface markers CD3, CD4 and CD8 were used to delineate T cell subsets in both BAL and blood. In paper I and II the study was done on samples from sarcoidosis patients, and staining of TCR AV2S3 was also performed in order to determine the frequency of T cells whose T cell receptors have this fragment in their variable region of the α chain. CD3, CD4, CD8 and TCR AV2S3 staining is part of the routine BAL evaluation which is performed on all sarcoidosis samples in Lung Research Lab. A CD4/CD8 ratio greater than 4 supports the diagnosis of sarcoidosis (179). Study of AV2S3 expression is one practical way to predict who are DRB1*03 positive before the results of HLA typing are obtained (180), since this is the case in virtually all patients who have an expansion of T cells with TCR AV2S3 in BAL (greater than 10.5% of CD4+ cells) (3 times the median frequency of TCR AV2S3+ cells in blood CD4+ T cells of normal healthy individuals). In paper II and IV all samples were stained with aqua fluorescent reactive dye (Live/Dead fixable dead cell stain kits, Invitrogen, USA) in order to discriminate between live and dead cells populations.

Intracellular cytokine staining was performed on both BAL and blood samples to evaluate cytokines and transcription factors. Following surface staining, fixation and permeabilization were performed on BAL and blood samples, thereafter intracellular staining was done for the cytokines and transcription factors of interest. Percentages of T cell subsets expressing the various markers were determined. The median fluorescence intensity (MFI) for cytokines and transcription factors was calculated in order to indicate the quantitative amount of each marker per cell (181). Matched isotype controls were used for each cytokine and transcription factor to discriminate specific antibody staining from non-specific background staining.

Elispot

In paper II, Elispot assay was performed on PBMC and BAL cells from sarcoidosis patients and on PBMC from healthy controls for detection of IFNγ and IL-17 following mKatG stimulation. Elispot plates were treated with 70% ethanol. Thereafter plates were coated with anti-IFNγ coating antibody or anti-IL-17 coating antibody (Mabtech, Sweden) overnight. After washing, plates were blocked with *complete tissue culture medium* (CTCM) for 2 h at room temperature and freshly obtained PBMC (3×10^5) or BAL cells (2×10^5) were added to each well. Alveolar macrophages of BAL samples were partly depleted by plastic adherence during one hour incubation before adding the samples to the Elispot plate. Cells were stimulated with 10 μg/ml mKatG. Phytohemagglutinin (PHA) and anti-CD3 monoclonal antibody were used as positive controls for IFNγ and IL-17 respectively and unstimulated cells were used as negative control. Cells were incubated at 37 °C in a humidified atmosphere

of 5% CO2 for 16 h during which secreted cytokines were captured by the coating antibodies. Subsequently, biotinylated IFNγ monoclonal antibody or biotinylated anti-IL-17 monoclonal antibody were added and samples incubated for 2 h, thereafter plates were incubated with streptavidin–alkaline phosphatase for 1 h. Finally substrate (BCIP/NBT, Mabtech) was added and plates were developed for 10–20 min. We applied extensive washing between each step and after the last washing and overnight drying, plates were scanned and counted using the Elispot Reader system with AID Elispot Software 4.0. Results are presented as a mean value of triplicate wells and normalized to spot forming cell (sfc) in one million cells.

Fluorospot analysis of IFNγ and IL-17 producing cells was done on some samples. Following labeling and coating of theIPFL plate©, cells werestimulated with mKatG. Secreted IFN-γ and IL-17 were captured and then stained with antibodies labeled with green and red fluorophore (FITC and Cy3). The plate was analyzed by an automated reader with filters for FITC and Cy3. The ability to detect cells that produce both cytokines simultaneously is the biggest advantage of Fluorospot compared to Elispot.

Quantitative immune PCR

The level (ie. concentration) of soluble IL-17 protein was measured in the cell-free BAL fluid of sarcoidosis patients with Löfgren's syndrome (DR3+ and DR3-), sarcoidosis patients without Löfgren's syndrome and healthy individuals. Due to the low absolute concentration of extracellular IL-17 protein in the diluted sample caused by the BAL procedure *per se*, we utilized a customized quantitative immuno-PCR (qIPCR) technology developed in A. Lindéns laboratory in collaboration with TATAA Biocenter™ (Göteborg, Sweden) (182). This qIPCR combines the high specificity of Enzyme-linked immunosorbent assay (ELISA) with the high sensitivity of quantitative PCR for detection of low concentrations of protein antigens (183). Plates were coated with anti-human IL-17 antibody and after blocking, 20 times concentrated BALF using ultrafiltration from each individual were added in triplicate. Thereafter each well was incubated with a detection IL-17 antibody/DNA conjugate (conjugated at TATAA Biocenter™; detection antibody from eBioscience™). A dilution series of recombinant human IL-17 was added to separate wells in triplicate and incubated in the same way as the samples for obtaining a standard curve. Real-time PCR was carried out by specific primers. After extensive washing, Ct values were determined for individual samples. The mean values of triplicate determinations were calculated after comparisons with a standard curve.

Immunohistochemistry

In order to visualize the expression of IL-17 in lung tissue from sarcoidosis patients we performed immunohistochemistry (paper III). Contiguous 4 μm thick transbronchial paraffin-embedded sections from sarcoidosis patients (patients with and without Löfgren's syndrome) and healthy controls were deparaffinised in xylene and rehydrated by gradient ethanol. Antigen retrieval was done by boiling slides in citrate buffer (pH 6.0). After elimination of the endogenous peroxidase activity in the 0.3% H_2O_2, blocking was done by 5% goat serum. Rabbit polyclonal anti IL-17 as primary antibody was used for IL-17 staining. The staining was performed in parallel with rabbit IgG isotype control and tonsil tissue was processed as the positive tissue control. Thereafter slides were treated with biotinylated secondary antibodies (goat anti-rabbit). The staining continued with Vectastain Elite ABC Kit and the immune reaction was visualized using 3,3′-diaminobenzidine.

Sections were then counterstained with Mayer's hematoxylin, dehydrated, mounted and viewed under light microscope at a magnification of ×100, ×200 and/or ×400.

Statistical analysis

Statistical analysis of the data was performed by GraphPad PRISM 5.02 (GraphPad Software Inc., San Diego, CA, USA). Mann–Whitney U test or unpaired *t*-test was used for comparisons between two groups (non-parametric or parametric values, respectively). Analysis of data for more than two groups was performed by one-way ANOVA test and followed by Dunn's or Tukey's post-test for non-parametric or parametric values respectively. Wilcoxon signed rank test was used for comparisons of dependent samples. Correlation analysis was performed by Spearman's rank correlation test. A *p*-value of less than 0.05 was considered to define statistical significance.

4 RESULTS AND DISCUSSION

4.1 Antigen-specific Th1 responses in sarcoidosis (Paper I)

Sarcoidosis is a multisystem granulomatous disease of unknown cause with commonly prominent lung involvement. Clinical manifestations of sarcoidosis vary from an asymptomatic state to a life-threatening condition (184). T cells are known to play a critical role in the pathogenesis of this disease and accumulation of activated CD4+ T cells in the airways of patients has been well documented. Given the increased levels of IFNγ and IL-2 in the lungs of sarcoidosis patients, it has been postulated that sarcoidosis is a Th1 mediated disease (185-187). However, accumulating data supports the importance also of other T cell subsets in the pathogenesis of sarcoidosis. The etiology of sarcoidosis is still unknown, although it has been proposed that mycobacterial species are involved in the pathogenesis of at least a subgroup of sarcoidosis patients (188). A specific mycobacterial antigen, mycobacterial catalase–peroxidase (mKatG) has been detected in sarcoidosis tissue. Furthermore, mKatG has been shown to trigger B and T cell responses in lung and blood cells of sarcoidosis patients (118-120). Here we wanted to investigate in detail Th1 and Th17 responses to mKatG in BAL and blood T cells from subgroups of sarcoidosis patients with distinct clinical manifestations.

Sarcoidosis patients enrolled in this study were classified as patients with Löfgren's syndrome (LS) and patients without Löfgren's syndrome. In addition, all patients with LS were selected to be HLA-DR3+ (DRB1*0301) and with an accumulation of $CD4^+$ TCR $AV2S3^+$ BAL T cells (n=13). In a sharp contrast all non Löfgren's syndrome patients should in addition fulfil the criterium to be HLA-DR3- (n=10). Since Löfgren´s syndrome in combination with HLA-DR3+ predicts the most favorable prognosis (140), we selected these two groups and hypothesized that they would reflect opposite ends of the "prognostic spectrum" in sarcoidosis. In particular HLA-DRB1*14 and –DRB1*15 alleles are strongly associated with a chronic disease course, at high risk of developing pulmonary fibrosis (141, 142).

IFNγ and TNF are important cytokines in mycobacterial clearance (189-191) and IL-2 is involved in T cell clonal proliferation (192). All three cytokines can be produced by CD4+ and CD8+ T cells. We selected these three cytokines to evaluate the production of each in response to mKatG in sarcoidosis patients.

Stimulation with mKatG led to activation of BAL and blood CD4+ T cell cytokine responses in both Löfgren's and non Löfgren's syndrome patients when compared to spontaneous cytokine production i.e. un-stimulated sample.

An important role of Th1 cells and related cytokines in protection against *M. tuberculosis* has been reported by different groups. Splenocyte T cells from (Bacillus Calmette–Guérin) BCG immunized mice could adoptively induce protection and control growth of *M. tuberculosis* in recipient mice (193). Although IFN-γ alone is insufficient to control *M. tuberculosis* infection, it is required for protection against *M. tuberculosis* and an early response of IFNγ producing T cells is critical to induce resistance to infection with *M. tuberculosis* (194, 195).

The requirement for TNF in control of *M. tuberculosis* infection is complex, e.g. it has a role as a mediator of macrophage activation. Moreover TNF in synergy with IFNγ induces nitric

oxide synthase-2 expression (Absence of NOS2 causes increased susceptibility to *M. tuberculosis* infection (196). TNF in *M. tuberculosis* infection is also involved in cell migration and cell localization within lungs. It also affects formation of functional granulomas in infected tissues by influencing the expression of adhesion molecules, chemokines and chemokine receptors (197).

CD8+ T cells in sarcoidosis patients exhibited a cytokine profile similar to CD4+ T cells following mKatG stimulation. Our data regarding responses of CD4+ and CD8+ T cells to mKatG is in accordance with a previous study showing positive IFNγ response after mKatG stimulation in U.S. patients (120).

Since MHC class I presentation is most efficient with cytoplasmic antigens, a possible role for $CD8^+$ T cells in the immune response to *M. tuberculosis* received little attention for many years, since mycobacterial antigens are normally restricted to phagosomes and presented on MHC class II to CD4+ T cells (197). However, mice with a genetic deficiency in β2 microglobulin, and thus deficient in MHC class I molecules were quite susceptible to *M. tuberculosis* infection (198). There is strong evidence that indicated migration of CD8+ T cells to the lungs in *M. tuberculosis* infection occurs with the same kinetics as that of CD4+ T cells (199) and these CD8+ T cells are capable to produce IFNγ and to lyse infected macrophages (200). Rapid accumulation of CD8+ T cells in the lung of mice infected with *M tuberculosis* has been reported before (201).

Both CD4+ and CD8+ T cells are capable to release cytokines and mediators to activate macrophages. Our data on the cytokine profile of CD8+ T cells indicated that CD8+ T cells not only contribute in cytotoxic activity but also that they are activated to cytokine production and to protect against *M. tuberculosis*. CD8+ T cells are found within granulomas and can help to prevent spreading of mycobacteria (202). Although the relative frequency of $CD8^+$ T cells in BAL of sarcoidosis patients is reduced at onset, their relative proportion increases at later stages of disease. Our data suggests that both CD4+ and CD8+ T cells play an important role in mycobacterial clearance, which is also supported by other studies (203).

Comparing T cell activity in response tomKatG between BAL and blood demonstrated that BAL T cells responded to mKatG with higher IFNΓ production than blood T cells in both Löfgren's and non Löfgren's patients. This indicated that mKatG specific T cells are accumulated in the affected organ. Moreover, it has been shown that alveolar T cells in sarcoidosis patients are more activated than peripheral cells (204).

We didn't compare BAL T cell reactivity to mKatG with irrelevant antigens in sarcoidosis patients. However, reactivity of BAL cells from sarcoidosis patients to mycobacterial antigen but not to Keyhole Limpet Hemocyanin has been shown by others (118, 205). Furthermore, T cell responses in blood of most sarcoidosis patients to mycobacterial antigen but not lysate from *Trypanosoma brucei* have been demonstrated by other groups (206).

In contrast to mKatG responses, IFNγ responses to PPD was observed only in CD4+ T cells, and not in CD8+ T cells, which indicates that there might be a selective recognition of the mycobacterial epitopes among T cells. Some mKatG-derived peptides may be constituents of PPD, but if so they would clearly form a small fraction of the whole protein mixture.

There were no major differences between patient subgroups regarding the production of individual cytokines in response to mKatG and PPD.

Among the T cells some can exhibit a multifunctional capacity, i.e. secrete two or more cytokines. As we mentioned before IFNγ and TNF are two important cytokines for combating mycobacterial infections. (189, 190, 194, 207). When combined, IFNγ and TNF synergize in their capacity to mediate effective killing (208, 209). There is some evidence that shows T cells secreting IFNγ, TNF and IL-2 simultaneously were found to possess the best protective capacity towards *Mycobacterium tuberculosis* (210).

We therefore investigated multifunctional T cells, i.e. CD4+ and CD8+ T cells that simultaneously produce two cytokines in response to mKatG and PPD. Among CD4+ and $CD8^+$ T cell subsets, we calculated the percentage of total mKatG-reactive cells that produced single IFNγ, single TNF, or IFNγ and TNF in combination (the same calculation was made for IFNγ in combination with IL-2). We found that mKatG stimulated the BAL CD4+ T cells to less single IFNγ production, but more simultaneous production of IFNγ and TNF, in patients with Löfgren´s syndrome as compared to non-Löfgren´s syndrome patients. In contrast, PPD stimulation gave rise to similar cytokine pattern in both patient subgroups. This finding suggests that DR3+ sarcoidosis patients with Löfgren's syndrome i.e. patients with favorable prognosis have a more potent immune response towards a minor number of antigens, in which mKatG could be one of them, which consequently could lead to antigen elimination followed by recovery. We may speculate that patients with non-Löfgren´s syndrome have a less potent response to mKatG and other antigens, allowing antigen persistence. Additionally, they may exhibit an epitope spreading within the lungs, leading to involvement of more antigens and more IFNγ production with solid inflammation and prolonged disease, since analysis of total CD4+ T cell IFNγ mRNA expression showed higher levels in DR3- patients (211).

The median fluorescence intensity (MFI) of cytokine staining was measured in patients with Löfgren´s syndrome. The MFI value is an indication of the quantity of cytokine content on a per-cell basis (212). Our findings demonstrated that the multifunctional CD4+ T cells i.e. IFNγ/TNF double producing cells, following mKatG stimulation, had the highest MFI values of each cytokine, suggesting that they produce more of the two respective cytokines from each cell, compared to single cytokine-producing CD4+ T cells and it indicates these multifunctional T cells have a more potent effector capacity with regard to each respective cytokine. Seder et al. have reported the importance of a potent and durable T cell response with a high frequency of T cells that are antigen specific in vaccine development (213). The frequency of IFNγ producing cells is the most common parameter used to evaluate vaccine responses due to the important role of IFNγ in pathogen clearance (190, 214). However, IFNγ-producing T cells are not sufficient for protection (215, 216) and presence of TNF is also important to induce protection. The role of TNF in immunopathology of tuberculosis is complex. Rheumatoid arthritis patients who were treated with anti TNF antibody were susceptible to developed fatal tuberculosis(217) which indicated the importance of TNF in protection against infection. The contribution of both IFNγ and TNF lead to enhanced killing of *M. tuberculosis* compared to either cytokine alone (208, 209, 218).

The importance of multifunctional T cells in immune reactions against tuberculosis (219), in BCG-vaccinated infants (220) and people who live in high infected areas (221) has been studied before. Multifunctional, high-level cytokine-producing Th1 cells in the lungs of mice have been associated with enhanced protection against *M. tuberculosis* (208, 210, 222). There is some evidence that protection against pathogens such as *Leishmania major* is associated with multifunctional T cells making IL-2, IFNγ and TNF (181). The induction of

multifunctional T-cells is a new approach in to the process of vaccine development (223, 224). We speculate that a high frequency of mKatG specific multifunctional T cells with a high per cell cytokine content may help clearance of the assumptive pathogenic antigen in sarcoidosis patients with Löfgren´s syndrome. Probably the potency of multifunctional T cells is due both to their double cytokine production, and the higher per cell cytokine content of each cytokine

As we described before sarcoidosis patients who are HLA-DR3+ most commonly have Löfgren´s syndrome and a good prognosis. The accumulation of AV2S3+ T cells in the lungs (>10.5% of total $CD4^+$ T cells) is an interesting characteristic of the patients in this group. TCR $AV2S3^+$ T cell proliferation of PBMC from healthy DR3+ BCG vaccinated individuals following *in vitro* stimulation with *Mycobacterium tuberculosis* extract has been shown by our group before (225). Our group in collaboration with John Hopkins University has reported a correlation between the frequency of BAL TCR AV2S3+ T cells and BAL IFNγ producing cells in response to mKatG by Elispot (120). In the current study we show, for the first time, the capability of BAL TCR AV2S3+ T cells to react against mKatG.

Our data demonstrated that TCR AV2S3+ T cells responded with IFNγ production to a significantly higher extent than the TCR AV2S3- T cells (median 0.65% vs. 0.48%, p=0.016). This finding indicates that mKatG could be a specific disease-related antigen that is capable to trigger different subsets of T cells. It is important to remember that mKatG is a large protein, approximately 700 amino acids, and likely contains several T cell epitopes that can be recognized by various TCRs. It is therefore not surprising that both TCR AV2S3+ and AV2S3- T cells can respond to the same protein.

Similar to our findings in BAL, the blood TCR AV2S3+ T cells also responded to mKatG to a higher extent than blood TCR AV2S3- T cells. We speculate that the TCR AV2S3+ T cells are re-circulating throughout the body. The alveolar T lymphocytes can reach regional lymph nodes by leaving the alveoli through the alveolar epithelium. Via the regional lymph nodes they can be distributed all over the body to rejoin the systemic immune system (226). Alternatively, the sarcoidosis antigen that stimulates the TCR AV2S3+ T cells may be distributed systemically.

Our group has previously shown that the frequency of CD4+ AV2S3+ T cells in BAL of patients after clinical recovery is normalized (180). This indicates that the number of BAL TCR AV2S3+ T cells correlates with disease activity. Furthermore we know that there is an association between the number of CD4+ AV2S3+ T cells and good prognosis (227). Previously, our group also reported that expression of activation markers such as CD26, CD28, CD69, and HLA-DR were enhanced in AV2S3+ BAL CD4 T cells compared to AV2S3- subsets. These data suggested that AV2S3+ CD4+ T cells in the lung are significantly more activated and differentiated compared to AV2S3- CD4+ lung T cells (228, 229). Moreover, in another study we showed a sharply reduced expression of the regulatory T cell transcription factor Foxp3 in BAL AV2S3+ CD4+ T cells of DR3+ sarcoidosis patients compared to AV2S3- cells (230) This indicates that BAL AV2S3+ CD4+ T cells are effector cells rather than regulatory T cells. These findings together with our observation of Th1 cytokine production by these cells and a higher response of TCR AV2S3+ T cells to mKatG compared to the other CD4+ T cells suggests that TCR AV2S3+ T cells are associated with good prognosis and spontaneously resolving disease because of their ability to secrete effector cytokines upon mycobacterial antigen stimulation, possibly leading to antigen elimination.

The TCR AV2S3+ T cell subset is not a T cell clone but it constitutes an oligoclonal T cell subset. The variable (V) α chain can associate with different Vβ chains. Most recently our group identified 16 different Vβ CD4+ T cell expansions in BAL of sarcoidosis patients and a preference of TCR AV2S3+ T cells to pair with Vβ22, Vβ7 and Vβ18 has been shown before using PCR (146, 231). We also have data based on flow cytometric analysis that indicates a preference for Vα2.3 to pair with Vβ22, although not in a majority of Va2.3+ cells (Kaiser *et al*, unpublished observations). T cell clones can exhibit different avidity towards a given antigen; one study showed that some clones only proliferated, whereas others both proliferated and produced cytokines, in response to a given concentration of the same antigen (232).

BAL T cells preferentially exhibited a CD27- phenotype while in blood the majority of CD4+ T cells were CD27+ T cells. Our findings thus revealed that differentiated T cells localized in inflamed organ. BAL CD27- T cells produced more IFNγ in response to mKatG, while in blood the CD27+ T cells were the major cytokine producing cells. Similarly in chronic beryllium disease (CBD), i.e. a granulomatous disease like sarcoidosis, characterized by infiltration of beryllium-specific CD4+ T cells in the lungs, the majority of cytokine producing T cells are CD27- (233). CD27, a co-stimulatory molecule (member of the TNF/NGF-R family), is expressed on the naïve and memory T cells. Activation of T cells via TCR/CD3 induces high CD27 surface expression. However, following prolonged activation, CD27 becomes gradually switched off (234-236). Our findings are therefore compatible with long-term antigen stimulation in the lungs.

4.2 IL-17 and antigen-specific IL-17-responses in sarcoidosis (Paper II)

T helper 17 is a subset of CD4+ T helper cells that less than a decade ago was identified as a distinct T cell lineage (237) and production of interleukin IL-17 (synonymous to IL-17A), a pleiotropic cytokine with widespread effects, is the hallmark of this subset of T cells (26). IL-17 is an essential player in host defense in several mammalian organs including the lungs of humans (182, 238). Th17 cells were recognized as a key factor in protection against extracellular bacterial and fungal pathogens like *Klebsiella pneumoniae* (239), *Citrobacter rodentium* and *Candida albicans* (240), while other studies showed the role of Th17 cells in protection against intracellular bacteria like *Mycobacterium tuberculosis* and *Salmonella enterica* (241). An impaired function of IL-17 results in increased bacterial burden and reduced overall host survival (242). IL-17 and IL-17-producing cells are thought to play a key role in chronic inflammation like mycobacterial infection, rheumatoid arthritis and other autoimmune disorders and also granuloma formation (243-245). Involvement of Th17 and Il-17 producing cells in inflammatory lung disorders such as asthma, COPD and ILDs have also been reported in several studies. Up-regulation of IL-17 has been reported in patients with asthma (246), although the exact role of IL-17 in asthmatic responses is difficult to define. It can be speculated that IL-17 in asthma doesn't have a prominent role and the influence of IL-17 in pulmonary inflammation is mostly restricted to neutrophil dependent disorders or Th2 independent chronic inflammation (247).

The involvement of IL-17 and IL-17-producing T cells in sarcoidosis has been reported by different groups, although there is some discrepancy regarding the role of IL-17 in pathogenicity of sarcoidosis. Facco et al. detected persistence of Th17 cells in both BAL and blood of sarcoidosis patients. They could also detect IL-17 producing cells surrounding the

central core of the granuloma from sarcoidosis patients. They also reported trafficking of Th17 cells to the lungs of sarcoidosis patients mediated by CCL29 (CCR6 ligand) (125). An increased population of Th17 cells in blood of sarcoidosis patients has been reported by Ten Berge *et al.* (126). They have also shown an increased proportion of IL-17/IFNγ double producing cells in BAL and blood of patients and increased numbers of IL-17 producing cells in and around granulomas. A significant correlation between IL-17 and IL-6 levels was detected by Urbankowski *et al*. in BAL of sarcoidosis patients (248). In contrast to Ten Berge, Furusawa *et al.* reported a decline in IL-17 mRNA level in PBMCs from patients with sarcoidosis compared to controls and they didn't observed any response to ESAT-6 stimulation (mycobacterial antigen) (249). Wonder Drake's group showed a greater number of Th17 cells in BAL and blood of sarcoidosis patients versus controls. In a sharp contrast with Furasawa's findings, they demonstrated an increase in ESAT-6 specific Th17 cell responses in BAL and blood of sarcoidosis patients compared to controls. Moreover they reported a reduction in expression of IFNγ by Th17 cells in sarcoidosis patients (250). Tondell et al. finally reported a lower fraction of Th17 cells in sarcoidosis patients compared to controls. However, they found a higher proportion of IFNγ producing Th17 cells in sarcoidosis patients and this was correlated with radiologic stage (251).

Our data reveals the presence of T cells making IFNγ as well as IL-17 in response to the mycobacterial antigen mKatG in sarcoidosis patients. mKatG-reactive IL-17 producing cells were found in the affected organ, the lung, as well as in peripheral blood. We and others previously showed the presence of mKatG-reactive IFNγ producing effector T cells in sarcoidosis patients (120). We have also reported a more pronounced IFNγ production by CD4+ TCR AV2S3+ T cells compared to CD4+ TCR AV2S3- T cells in response to mKatG (252). Altogether these findings indicated mKatG behaves in a manner expected for a pathogenic antigen.

Here for the first time, we have shown mKatG-specific IL-17 responses in BAL of sarcoidosis patients with higher frequency of mKatG-reactive IL-17 producing cells in BAL of sarcoidosis patients with Löfgren's syndrome compared to patients without Löfgren's syndrome (Figure 5a). Furthermore sarcoidosis patients with Löfgren´s syndrome and more precisely sarcoidosis patients with Löfgren´s syndrome who are DR3+ (patients with accumulation of CD4+ TCR AV2S3+ T cells in the lungs) had higher levels of IL-17 in BALF (Figure 5b). Importantly this pioneer finding could be a validation for the pathogenic role of mKatG in a subset of sarcoidosis patients. The specific capacity of the HLA-DR3 molecule in presenting sarcoidosis-specific antigen(s) such as mKatG for T cells followed by induction of Th17 cells in the lungs of DR3+ sarcoidosis patients with Löfgren's syndrome can be a possible explanation for higher levels of soluble IL-17 in this subset of patients.

Our findings demonstrated that mKatG-reactive IFNγ producing cells are dominant compared to mKatG-reactive IL-17 producing cells in BAL of sarcoidosis patients. However, given the importance of Th17 and IL-17 producing cells in the immunopathogenesis of several inflammatory and autoimmune diseases (253), we propose that the observed Th17 responses against the mycobacterial protein mKatG could contribute to the inflammation in sarcoidosis. The accumulation of IL-17 producing cells reactive to mKatG in the lungs of sarcoidosis patients may be a result of trafficking or local proliferation of antigen-specific IL-17 producing cells at the site of granulomatous inflammation.

The finding of more IL-17-producing cells responding to mKatG in sarcoidosis patients with Löfgren´s syndrome locally at the site of inflammation suggests further qualitative differences in the mKatG-specific responses between patients with and without Löfgren´s syndrome. Due to the very good prognosis in DR3+ sarcoidosis patients with Löfgren's syndrome we postulate that IL-17-producing cells play a role in the presumed elimination of antigen and spontaneous recovery that is characteristic for DR3+ patients with Löfgren´s syndrome.

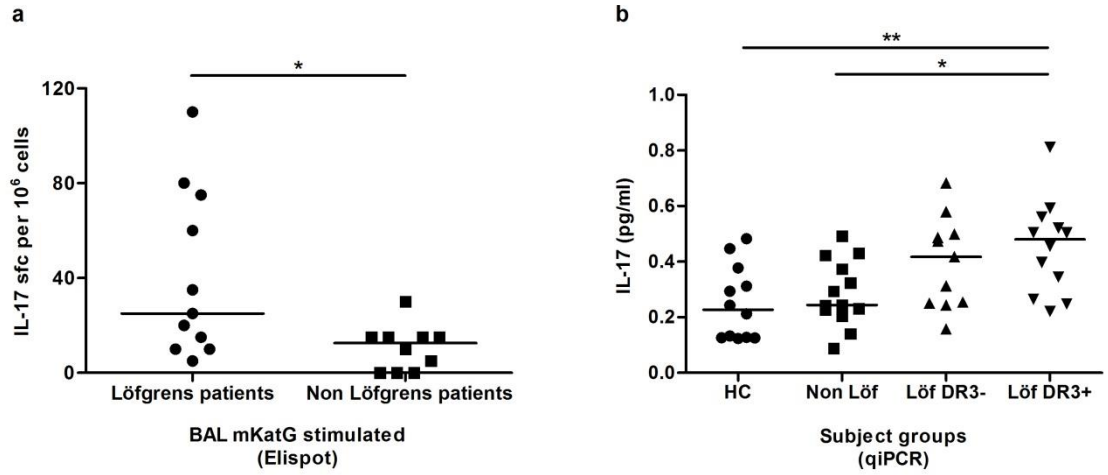

Figure 5: mKatG-specific IL-17 responses and IL-17 levels in patients with pulmonary sarcoidosis. (a) Elispot assay for evaluation of mKatG specific IL-17 producing cells in BAL of patients with and without Löfgren´s syndrome. (b) IL-17 levels in BAL fluid determined by Immuno-PCR and compared in healthy controls, sarcoidosis patients without Löfgren's syndrome and with Löfgren's syndrome. Sarcoidosis patients with Löfgren´s syndrome were divided into two groups (HLA-DR3+ and HLA-DR3-).

Both Th1 and Th17 cells are required and interact in different steps for host defence against mycobacterial infection. In the lungs of vaccinated animals after *M. tuberculosis* challenge, expression of the CXCR3 ligands i.e. CXCL9, CXCL10 and CXCL11 have been induced by IL-17, thereby IL-17 can regulate trafficking of Th1 cells to the site of infection (241). IFNγ and IL-17 T cell responses to *M. tuberculosis* antigens are time dependent and IL-17 producing T cells accumulate in the lungs more rapidly than IFNγ-producing T cells; furthermore IFNγ and IL-17 may act in synergy to initiate inflammation (254). IL-17 stimulates the activity of the transcription factor NF-κB (255); activation of NF-κB in antigen presenting cells in turn affects Th1 responses and leads to development of IFNγ producing T-cells (256). IL-17 is not only involved in neutrophil-mediated inflammatory responses, but also it is an important cytokine in the induction of an optimal Th1 response and protective immunity against mycobacterial infection. We speculated that the higher expression of IL-17 and mKatG specific IL-17-producing cells in sarcoidosis patients with Löfgren's syndrome help to induce Th1 responses, and contribute to a more rapid and diverse effector T cell response, which then contributes to the protection against unknown antigen(s).

The inflammatory properties of IL-17 and IL-17 producing cells are irrefutable and involvement of IL-17 in the pathogenesis of several inflammatory disorders, in particular autoimmune diseases such as rheumatoid arthritis (RA), multiple sclerosis (MS), psoriasis and inflammatory bowel disease (IBD) has been reported before (19, 257). IL-17 has been

considered as a potent pro-inflammatory cytokine that induces expression of different inflammatory mediators such as IL-6, TNF-α, IL-1β, GM-CSF, CXCL1, CXCL8, CCL2, CCL7 and CCL20 by different cell types like epithelial cells, endothelial cells and macrophages (258). Th17 and possibly other IL-17 producing cells in sarcoidosis patients can be considered as potent mediators of inflammatory responses because of their ability to induce trafficking and activation of inflammatory innate immune cells at the site of inflammation (259, 260).

More recently there is some evidence for an immune-modulatory or non-pathogenic role for IL-17. The role of IL-17 and antigen specific IL-17 producing cells in the pathogenesis of experimental autoimmune uveitis (EAU) has been demonstrated before (261). Yan Ke *et al.*, however, identified an anti-inflammatory role for IL-17 in the EAU model. They found that in EAU-susceptible rats, treatment with small doses of IL-17, rather than exacerbating the clinical score of the disease, lead to suppression of EAU development and unexpectedly ameliorated the clinical score of EAU. They thus concluded that IL-17 has both pro- and anti-inflammatory effects on the development of EAU (262). O'Connor *et al* have demonstrated a protective function for IL-17 in a mouse IBD model and suggested that this function is exerted partly by suppressing Th1 differentiation. They also showed that absence of IL-17 led to an accelerated and severe disease accompanied by higher expression of genes encoding Th1 type cytokines (263). Thus, we cannot exclude this type of beneficial effect of IL-17, particularly in the DR3+ Löfgren´s patients.

Another recent and intriguing finding is that Th17 cells can be polarized to become pathogenic or non-pathogenic depending on the environmental cytokines or mediators (22). Kuchroo's group has shown a critical role of TGF-β in development of Th17 cells. They reported that although the presence of IL-23 has been considered to be essential for developing the pathogenic Th17 phenotype, TGF-β3 can differentiate naïve T cells into pathogenic Th17 cells without any need for further exposure to IL-23. In fact both TGF-β1 and TGF-β3 are able to induce Th17 cells, but Th17 cells induced by TGF-β1 are not pathogenic whereas TGF-β3 has an important role in induction of pathogenic and pro-inflammatory Th17 cells. We know from previous studies that the levels of some cytokines like TGF-β1 that may influence differentiation of Th17 cells, differ between sarcoidosis patients with or without Löfgren's syndrome (211).

Our findings indicate that in sarcoidosis patients, there is a subset of T cells which has the ability to produce both IFNγ and IL-17 simultaneously. In fact, we found that a majority of T helper 17 cells can also produce IFNγ. Existence of these hybrid T cells has been reported before in other inflammatory diseases and also sarcoidosis (126). Monteleone et al. demonstrated that as a consequence of cytokines and microenvironmental molecules in gut mucosa of IBD patients, conditions are generated that result in that some IFNγ producing cells originate from previously IL-17 producing T cells (264). Conversion or switching of T helper cells from IL-17 producing to IFNγ producing cells is a phenomenon that is dependent on the cytokine environment. IL-1, IL-6 and TGF-β have important roles in the cytokine signature of Th17 and IL-17 producing cells. TGF-β is essential for sustained expression of IL-17A by Th17 cells. In the absence of TGF-β, both IL-23 and IL-12 cause suppression of IL-17 expression and instead enhance IFNγ production (265). Importantly, there is evidence that hybrid Th1/Th17 T cells are more pathogenic than conventional Th17 cells (266). CD4+ AV2S3+ T-cells and CD4+ AV2S3- T-cells displayed differences with regard to the capacity

to express IFNγ and IL-17. However, further studies are needed to investigate the frequencies of such hybrid T cells in patient subgroups versus healthy control subjects.

In most cases the first option for reducing the inflammation in sarcoidosis patients is to prescribe corticosteroids. In recent years, the blocking and controlling of IL-17 and IL-17 producing cells is one of the strategies that have been used for treatment of inflammatory disorders, for instance administration of antibody against IL-17 described in RA and psoriasis (267, 268). On the other hand, a protective effect of IL-17A in inflammatory bowel diseases (IBD) and uveitis has been reported before (24, 262). Given our findings that indicated higher level of IL-17 and increased IL-17 responses among a subgroup of sarcoidosis patients with good prognosis, it remains to be elucidated whether inhibiting this signaling will offer clinical benefits for patients. Therefore, a more detailed understanding about the pathogenic or protective role of Th17 cells in various subsets of sarcoidosis patients is needed.

4.3 Characteristics of effector and regulatory T cell subsets in the lungs of smoking and non-smoking healthy individuals (Paper III)

Cigarette smoke intrinsically induces local inflammation; however, it is associated with both release and inhibition of pro-inflammatory and anti-inflammatory mediators that influence different T cell subsets. T helper lymphocytes have a pivotal role in orchestrating the host defense and inflammatory reaction following triggering by specific antigens. Our group has demonstrated an impact of smoking on the distribution of BAL T cell subsets in the context of COPD studies (269). In the present study, in order to better understand the influence of cigarette smoking on T cells in relation to the pathogenesis of smoking-induced diseases, we investigated frequencies and characteristics of lung and blood CD4+T cell subsets such as T helper 1 (Th1), Th17 and Treg cells (and corresponding subsets in CD8+ T cells) in 18 healthy young and moderate smokers and 15 never-smoking individuals.

Our findings indicated a lower CD4/CD8 ratio of BAL T cells in healthy smokers. A higher rate of apoptosis in alveolar cells and alterations in glycoproteins in smokers can be possible explanations for changing this ratio in BAL and blood. Cigarette smoke components and even the gaseous phase can directly induce both apoptosis and necrosis of lymphocytes at the site of exposure (270, 271).

Our data demonstrated that healthy smokers had a lower frequency of BAL CD4+ IL-17 producing cells compared to healthy never smokers. Inflammatory responses can be induced by smoking and involvement of IL-17 producing cells in this has been reported before (158, 245). A higher frequency of IL-17 producing cells in the bronchial biopsies of COPD patients compared to control non-smokers has been documented. Although COPD patients are different from healthy smokers with normal lung function, studies on the role of IL-17 in COPD patients can be informative.

COPD is an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases in particularly cigarette smoke and is characterized by persistent and progressive airflow limitation (272). Increased production of IL-17A in the bronchial submucosa and infiltrating inflammatory cells in small airways has been reported (273, 274). Chang et al. reported expression of IL-17A by both CD4 and CD8 T cells in the lung submucosa from COPD patients and suggested that IL-17 producing cells play an important role in the pathogenesis of COPD by inducing an accumulation of neutrophils in the lungs of

COPD patients (275). Airway epithelial cells were induced by IL-17 to produce mucus and matrix metalloproteinase-9 (MMP-9) and dysregulation of MMPs contribute to the destruction of lung tissue in COPD (276). Neutrophils can contribute in the pathogenesis of COPD by secretion of proteolytic enzymes such as neutrophil elastase (277). Elastin is a major constituent of the extracellular matrix in the lungs and it can be degraded by neutrophil elastase, leading to mucus hyper-secretion (278). Moreover it has been suggested that elastin can act as an auto-antigen in the lung of COPD patients by inducing of Th1 and Th17 cells against elastin (276). It has been suggested that in COPD patients with frequent exacerbations and excessive mucus production, these phenomena can be attributed to IL-17 mediated neutrophilia (245). Shen et al. has demonstrated not only that the concentration of IL-17 and neutrophil percentage decline in the lungs of tobacco-smoke-exposed mice which were treated with anti-IL-17 antibodies, but also the pathological score of small airway inflammation was improved (279). This indicated the importance of IL-17 in the pathogenesis of COPD. Doe et al. demonstrated a similarity in IL-17 expression between COPD patients and healthy smokers but no association with increased neutrophilic inflammation (280).

There is no consensus on the effect of smoking on IL-17 producing cells in healthy smokers with normal lung function and this is partly related to variations in methodology and differences regarding the characteristics of studied populations. Tobacco smoking can induce an antigen specific Th17 response in certain individuals (281) and higher relative expression of IL-17 mRNA in lung tissue of smokers has been reported before (282). Our finding regarding the frequency of BAL CD4+ Th17 cells are in contrast with these studies. We found a reduced IL-17 expression by BAL CD4+ T cells of light smokers but an increase in the number of cigarettes per day caused an increased IL-17 expression. We should emphasize that most studies related to the effect of smoking on T cells focused on differences between never-smokers and smokers who were matched with COPD patients with regard to smoking history, while the present study was performed on samples from mild smokers.

The frequency of IFNγ-producing CD8 T-cells increased in BAL of healthy smokers after anti-CD3/CD28 stimulation and this increase was more intensive in heavy smokers. Freeman et al. demonstrated increased production of inflammatory cytokines by CD8+ T cells in COPD patients.The lung CD8+ T cells respond to danger signals by up-regulating inflammatory mediators and cytotoxic molecules (283, 284). IFN-γ is a key cytokine in the development of the airway obstruction and pulmonary destruction in COPD by induction of different mediators (285).

Bacterial burden and susceptibility to develop some infections increase among smokers (286). Cigarette smoke exposure can inhibit T cell responses to *M. tuberculosis* and influenza virus in a mouse model (287). Lugade et al. demonstrated a negative effect of cigarette smoke exposure on the generation of adaptive immune responses to nontypeable *Haemophilus influenzae* (NTHI) (288). Although in contrast to our findings they reported increased IL-17 response and decreased IFNγ response to NTHI chronic infection in mice with chronic cigarette smoke exposure. There may however be several reasons for this discrepancy, such as species differences, the level of smoke exposure, infection in mice versus in vitro stimulation of human T cells with anti-CD3 antibodies. Due to the important role of Th17 cells in host defense against bacterial or viral infections, we propose that the decline in expression of IL-17 in smokers can increase infection susceptibility.

The multifunctional T cells (cells that produce two or more cytokines simultaneously) as well as hybrid cells (T-cells which are capable to produce cytokines from two different T cell lineages) have been studied in BAL and blood of healthy smokers and non-smokers. The multifunctional IFNγ/TNF producing cells are associated with either a protective role or are indicative of active disease in tuberculosis (289-291). IFNγ/IL-17 producing T cells is an example of hybrid T cells and indicates plasticity between Th1 and Th17 cells. Less is known about the function of these hybrid T cells but there is some evidence to suggest that Th17/Th1 T cells are more pathogenic than conventional Th1 or Th17 cells in the context of autoimmunity (266, 292). We believe these multifunctional or hybrid cells are potent functional T cells which play an important role in host defense. Our findings demonstrated a decline in BAL CD4+ IFNγ/IL-17 and IL-17/TNF producing cells in healthy smokers compared to never smokers. The reduction of these potent T cells could negatively impact on the capacity of the immune system in smokers to control pulmonary infections.

Our findings on regulatory T cells (Treg) showed no difference in the proportion of Foxp3+ CD4+ T cells in BAL of smokers and never-smokers. Similarly to the situation for IL-17, there is no consensus on the impact of smoking on Treg cells and an augmented or abated frequency of FoxP3 expression (293, 294) and even variation between smokers and non-smokers in different compartments of lung tissue has been reported. Isajevs et al. have demonstrated increased Foxp3 expression in large airways of smokers and COPD patients but a decreased percentage of Foxp3+ cells in small airways of COPD patients (295). Furthermore, we found that Foxp3+ CD4+ regulatory T cells in BAL of smokers did not show any IL-10 production (Figure 6a). IL-10 is a suppressive cytokine that hinders transcription of many genes which are normally induced via pattern recognition receptors. Many immune cell types including effector or regulatory T-cells can produce IL-10 (296).

IL-10 can be produced by Foxp3+ Treg cells in tissues such as colon or lungs and it is needed for restraining immunological hyperreactivity at these interfaces with the environment, but is not needed to control systemic autoimmunity (297). We speculate that the lack of IL-10 production by Foxp3+ CD4+ regulatory T cells in BAL of smokers may reflect a defective function of regulatory T-cells which is caused by cigarette smoke components. Roos-Engstrand *et al*. have shown that despite similar frequencies of T cells expressing Foxp3 in smokers and non-smokers, the numbers of non-functional Tregs with a CD4+ CD25+ phenotype that do not express Foxp3 is higher in healthy smokers compared to non-smokers (298).

Tregs constitute a heterogeneous population with diverse sub-populations, e.g. thymus-derived Tregs (tTregs), and peripherally derived Tregs (pTregs). These Treg variants have different functions and it has been proposed that tTregs modulate T-effector cell trafficking, while pTregs inhibit T-effector priming (37, 44). Helios is a member of the Ikaros transcription factor family and recently has been considered as a marker of natural or thymic-derived Treg cells (38), although this has later been challenged (39) and more recently, Helios has been suggested to be a marker of anergic effector cells or a T cell activation and proliferation marker (40, 41). Regardless of Helios qualification as a tTreg or activation marker our findings indicate a remarkable increase in the proportion of Helios- Foxp3+ Tregs in healthy smokers (Figure 6b). Raffin *et al.* found that Helios^{-} Tregs, but not Helios^{+} Tregs has capacity to secrete IL-17 and IL-10 (299). In agreement with their findings, our data indicated that expression of IL-10 and IFNγ in Foxp3+ Tregs was almost exclusively confined to the Helios- subset. Moreover we found that the fraction of IL-10 positive cells in

the Helios- Tregs was significantly reduced in smokers (Figure 6c) and no such difference was observed for IFNγ production. The lower expression of IL-10, but not IFNγ, in Foxp3+Helios- BAL Tregs of smokers may indicate that smoking induces a defective function in Foxp3+Helios- Tregs. Alternatively, if Helios- Treg cells remain potent suppressors, the observed decrease in Th17 cells and other inflammatory cytokine producing cells in smokers may be partly ascribed to a high frequency of these Helios- Tregs in smokers. Such a scenario would be in accordance with Raffin's finding that demonstrated Helios- Tregs have higher suppressive capacity versus to Helios+ Tregs (299). Our finding of a reduced ratio of IL-17- to IL-10-producing CD4+ BAL T cells in healthy smokers is in contrast to the findings in smoke-exposed mice by Wang, although their model was based on heavy smoke exposure that induced a COPD-like disease (300).

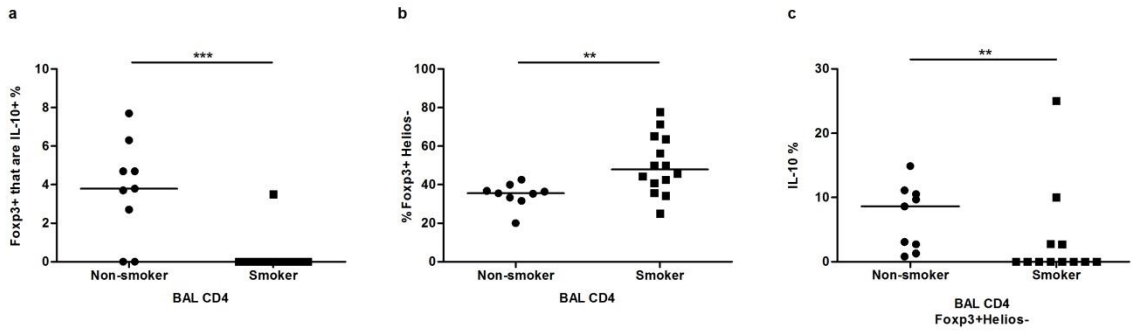

Figure 6: Analysis of regulatory T cells, (a) comparison of the frequency of BAL CD4+ Foxp3+ T cells that produce IL-10 between smokers and non-smokers. (b) Comparison of fractions of BAL CD4+ Foxp3+ T cells that are Helios- in smokers and non-smokers. (c) Comparison of IL-10 production by Foxp3+Helios- BAL CD4+ T cells between smokers and non-smokers.

There are discrepancies between different studies as to the effect of smoking on T-cell responses and several explanations are plausible. Daily quantity of exposure, smoking history, intensity of smoking inhalation varies between different smokers and those are just some of the factors that have an influence on T-cell function studies. Genetic background and socioeconomic status are other factors that contribute to the results. Whether studies were performed on human or mouse samples, and design of study *ex vivo* or *in vitro* as well as the choice of experimental techniques are other factors that affect the T-cell responses observed.

In conclusion, our findings implied that a change in subset composition of Tregs and reduced IL-10 production by Tregs may be of importance for induction of smoking-induced diseases such as COPD and autoimmune disorders.

4.4 Characteristics of lung T cell subsets in multiple sclerosis patients (Paper IV)

Cigarette smoke is recognized as a major risk factor for developing several diseases. In addition to the lung related disorders smoking has also been associated with risk of disease or to disease phenotype in a number of important autoimmune disorders (151, 301).

Rheumatoid arthritis (RA) is one of the immune mediated diseases with a striking example of gene-environment interaction. Certain variants of HLA–DRB1*01 and HLA–DRB1*04 are called the shared epitope genotype. A study on RA patients demonstrated a gene-environment interaction as the combination of smoking history and having two copies of HLA-DR "shared epitope" genes increased 21-fold the risk of the RA in the subgroup of patients who are positive for antibodies to citrullinated protein antigen (ACPA) (162, 302). Part of the mechanism appears to be that cigarette smoke induces the PAD enzymes responsible for citrullination (a post-translational modification) of proteins such as vimentin (163).

MS is another example of autoimmune disease where cigarette smoking strongly increases the risk of developing MS in people with genetic susceptibility, in this case carriage of HLA-DRB1*15 and absence of HLA-A*02 (171). Furthermore Odoardi et al. by study on Lewis rat transfer experimental autoimmune encephalomyelitis (EAE, a model of MS) have recently demonstrated that the lungs can contribute to the activation of potentially autoaggressive T cells and their transition to a migratory mode as a prerequisite to entering their target tissues and inducing autoimmune disease (303) and they concluded that T cells become educated in the lung to enter to the central nervous system.

T cells play an important role in orchestrating inflammation. However, very little is known about how smoking may lead to autoimmunity. To better understand the mechanisms behind the T cell activation in the lungs we investigated frequencies and characteristics of lung and blood CD4+ and CD8+ T cell subsets in seventeen MS patients (8 smokers and 9 non-smokers) (10 treated with IFNβ, 3 with natalizumab and 4 untreated patients) and twenty three healthy individuals (14 smokers and 9 non-smokers). IFNβ and natalizumab are two common, and the most effective, treatments in MS patients.

Pulmonary complications are reported in some MS patients, caused by pulmonary muscle dysfunction and destruction of cranial nerves (304). We didn't find any pulmonary complications in our patients and all enrolled patients had normal lung function.

Increased level of inflammatory cytokines such as IFNγ and IL-17 in CNS and blood from MS patients has been well documented (305-307). A dominant response of either Th17 or Th1 type has been reported to be associated with differences in preferential involvement of brain or spinal cord respectively and disease phenotype (166, 308). Our findings regarding BAL T cell cytokine production demonstrated that IL-17 production in BAL CD4+ T cells declines in MS patients regardless of smoking status and this effect is probably related to the treatment. The suppression of IL-17 production by IFNβ has been reported before and it has been suggested that this effect is exerted via induction of IL-27 secretion by dendritic cells (309, 310).

Sweeney et al. reported a correlation between reduced frequency of Tregs and severity of clinical symptoms of MS patients (310). Moreover, there are some studies that show defective suppressive function of Tregs in MS patients (43). Treatment with IFNβ leads to up-regulation of GITRL on dendritic cells and down-regulation of CTLA-4 on Tregs. CTLA-

4 induces negative signal into T cells to inhibit T cell proliferation, while GITR delivers costimulatory signals to induce T cell proliferation. Thus up-regulation of GITRL on dendritic cells and down-regulation of CTLA-4 on Foxp3+ T cells could provide a signal to motivate proliferation of regulatory T cells. It has been shown that there is an increased frequency of Foxp3+ Tregs and a partial improvement of Treg suppressive function in MS patients treated with IFNβ (311, 312). In accordance with other studies, we detected no significant difference in the frequency of Foxp3+ CD4+ T cells in BAL and blood of MS patients compared to controls (313). However, IFNβ treated patients have the highest frequency of Foxp3+ cells among our patients. We believe that the similarity in frequency of Foxp3+ T cells between patients and controls is likely related to the effect of IFNβ treatment. We propose that an increased frequency of BAL Foxp3+ T cells in both natalizumab and IFNβ treated patients and decrease in IFNγ and IL-17 producing cells in BAL of treated patients might indicate that treatment with IFNβ or natalizumab restored the frequency and function of Foxp3 regulatory T cells in BAL of patients, but the reason for this effect to occur in the lung and not in peripheral blood remains to be addressed.

Foxp3 instability and Treg plasticity is another reason for impaired function of Tregs. Komatsu *et al.* demonstrated conversion of Foxp3+ Tregs into Th17 cells leading to increased numbers of pathogenic Th17 cells in arthritis and IL-17 producing exFoxp3 cells have pathogenic function with higher affinity to self-antigens (314). Dominguez *et al.* reported higher frequency of IFNγ producing Foxp3+ T cells, i.e. Th1-like Tregs, in untreated MS patients. These Th1-like Tregs are regulatory T cells affected by functional plasticity with reduced suppressive function (315) and after treatment with β-interferon, their frequency were normalized in MS patients. In accordance with findings of Dominguez, we did not detect any differences in BAL Th1-like Tregs between MS patients and healthy controls since the most of enrolled patients were treated. However, we found that these plastic Tregs in BAL were affected by environmental signals since the frequency of Th1-like Tregs declined in both healthy smokers and MS smokers compared to non-smoking individuals.

IL-10 is a regulatory cytokine expressed by numerous cell types. It is a potent anti-inflammatory cytokine, which through its suppressive effects on many cells inhibits various inflammatory pathologies (316). IL-10-producing CD4+CD25+ T cells are present mainly within the intestinal lamina propria and resolution of murine colitis is dependent on the presence and enrichment of IL-10-producing CD4+CD25+ T cells in the intestine (317). Ranatunga et al. described that colitis resistance is associated with accumulation of IL-10 producing Foxp3+ T cells within the lamina propria. They also reported that IL-10 producing cells control expression of inflammatory cytokines and reduce the accumulation of pathogenic Th17 cells in the gastrointestinal tract (318). IL-10 has also been found to be of importance for immune regulation at the epithelial surfaces in the lungs. It has been reported that lung CD4+CD25+Foxp3+ T cells from naive mice can reduce airway hyper-responsiveness by a mechanism dependent on the induction of both IL-10 and TGF-β production (319, 320). Our findings demonstrated a down-regulation of IL-10 production by Foxp3+ Tregs in BAL of both healthy smokers and MS patients (regardless of smoking status). Thus, smoking-induced impairment of Treg function may be one factor that promotes autoimmunity, but several other mechanisms likely can contribute to such immune dysregulation. It remains to elucidate the exact role of IL-10 producing CD4+ Foxp3+ regulatory T cells in MS patients.

Regulatory T cells play a critical role in immune homeostasis and Foxp3 is considered as a master transcription factor in the development of Tregs. Tregs can be categorized into two main subgroups: natural regulatory T cells (tTreg) and adaptive regulatory T cells (pTreg) (321). tTregs that develop in the thymus and their main responsibility is to prevent autoimmunity in response to self-antigens, whereas pTregs are generated in the periphery in response to antigen stimulation and their function is to control and suppress T cell activity against foreign or neo-antigens (322). Helios is another transcription factor that plays an important role in the function of regulatory T cells. Raffin *et al* demonstrated that Helios-Tregs are potent regulatory T cells with higher suppressive capacity (299). They suggested Helios can be a marker of tTregs, although the validity of this is controversial, and alternatively Helios has been considered as an activation marker. They have shown that Foxp3+/Helios- Tregs have capacity to secrete IL-17 and IL-10. Moreover, these cells lose suppressive function under specific circumstances and switch to a pro-inflammatory phenotype. Our study demonstrated an increase in the frequency of Foxp3+Helios- Tregs and a corresponding decrease in Foxp3+Helios+ Tregs in BAL of MS patients. Thus we could detect in the BAL of MS patients a down-regulation of a Treg subset which is associated with control on self-reactive T cells and with a stable suppressive phenotype. Moreover we found an increase in the frequency of Foxp3+Helios+ Tregs in IFNβ treated patients that might be one of the main benefits of IFNβ treatment.

Finally, the balance between Treg and Th17 cell subsets plays an important role in the development of autoimmune and inflammatory diseases (323). A high frequency of IL-17 producing CD4+ T cells (307) and low frequency of Foxp3+ T cells have been reported in MS patients with active disease (324). In contrast, we found an increase in the ratio of Foxp3/IL-17 cells in both BAL and blood of non-smoking MS patients compared to healthy controls and this ratio was elevated in patients who treated with β-interferon. The Foxp3/Th17 ratio was increased in patients treated with β-interferon, mainly because the treatment caused an increased frequency of Foxp3+ T cells. However, we believe that the notion of a Treg/Th17 imbalance may be too simplistic to explain important features of the immunopathogenesis of MS, and the characteristics of Th17 and Treg cells need to be considered separately.

5 CONCLUSIONS

- There was a higher frequency of mKatG reactive IFNγ producing CD4+ T cells within the AV2S3+ subset compared to AV2S3- cells of both BAL and blood from HLA-DR3+ sarcoidosis patients with Löfgren's syndrome and a lung accumulation of TCR AV2S3+ CD4+ T cells.

- Sarcoidosis patients with Löfgren's syndrome had higher proportions of multifunctional cytokine producing T cells (IFNγ /TNF) in response to mKatG compared to patients without Löfgren's syndrome.

- Patients with Löfgren´s syndrome had a significantly higher frequency of IL-17-producing cells in BAL following mKatG stimulation, compared to patients without Löfgren´s syndrome.

- HLA-DR3+ sarcoidosis patients with Löfgren´s syndrome had a significantly higher concentration of IL-17 in BAL fluid compared to healthy controls and patients without Löfgren's syndrome.

- BAL CD4+ T cells in healthy smokers with normal lung function have significantly lower frequencies of IL-17 producing cells compared to healthy never smokers.

- IFNγ/TNF double-producing BAL CD4+ T cells had a significantly higher per cell content (MFI value) of both IFNγ and TNF compared to cells that produced either of the cytokines alone. This was observed in both sarcoidosis patients and healthy individuals.

- IL-10 expression was reduced in BAL CD4+ Foxp3+ T regulatory cells from smokers with normal lung function compared to healthy never smokers.

- The fraction of Foxp3+/Helios- Tregs was higher in healthy smokers compared to never smokers and IFNγ and IL-10 cytokine production in both smokers and non-smokers was mainly confined to Foxp3+/Helios- Tregs subset.

- There was a significantly lower ratio of IL-17+ to IL-10+ cells in BAL CD4+ T cells in healthy smokers compared to never smokers.

- The cytokine pattern of MS patients indicated a sharp down-regulation of IL-17 expression by BAL CD4+ T cells in MS patients who received β-interferon or natalizumab compared to patients who didn't received any treatment.

- MS patients who were treated with β-interferon or natalizumab had a tendency toward higher percentages of Foxp3+ CD4+ T cells in BAL compared to untreated patients. MS non-smokers and MS smokers had significantly fewer IL-10 expressing Foxp3+ cells compared to healthy non-smokers.

- The proportion of Foxp3+ BAL CD4+ cells that are Helios+ declined in MS patients, while patients who received β-interferon had a tendency towards higher relative frequency of Foxp3+ Helios+ cells in the BAL CD4+ T cell compartment.

6 CONCLUDING REMARKS AND PERSPECTIVES

The observed heterogeneous responses to mKatG in sarcoidosis patients strengthen the hypothesis that mKatG (and other mycobacterial antigens) are pathogenic antigens in a large group of patients, and that the quality of the T cell responses against such antigens may determine disease outcome. An important question for future studies to address will be to find out if there is a way to enhance multifunctional T cell or IL-17 responses, and if so is it beneficial at least in a subgroup of sarcoidosis patients? Finding dominant peptide epitopes of mKatG in the context of different HLA types is another important task that should shed light on the precise pathogenic mechanisms. In the future, antigen-specific therapy with T cell epitopes may be possible for patients with non-resolving disease.

If the lungs are involved in initiation and also propagation of the inflammatory process in MS, alterations of BAL Treg phenotype may be of relevance for the pathogenesis. The observed effects of treatment on frequency and function of lung Treg cells, and the Treg/Th17 ratio in IFNβ-treated patients, may be parts of the mode of action whereby this treatment leads to disease amelioration in MS patients. Important questions that remain to answer are: what are the functional consequences of the elevated frequency of Foxp3+/Helios+ Tregs in IFNβ-treated MS patients? What are the relative roles of different Treg subsets in the suppression of auto-reactive T cells? How much similarity is there between phenotype and function of the lung and CNS T cells and effect of treatment on Treg phenotype in different organs considering that the CNS is the site of auto-aggressive attack? What is the role of the lung in the induction versus the regulation of autoimmunity, not only in MS but also in other autoimmune disorders such as RA? Further studies of lung T cells in MS should help to better understand the immunopathogenesis of this disease, and may suggest new approaches to control the inflammatory process.

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8 REFERENCES

1. Murphy K, Travers P, Walport M, Janeway C. Janeway's immunobiology. 8th Edition ed; 2012.
2. Judy Owen JP, Sharon Stranford. Kuby Immunology. 2013.
3. Abbas AK, Lichtman AH. Basic immunology: functions and disorders of the immune system; 2011.
4. Meierovics A, Yankelevich WJ, Cowley SC. MAIT cells are critical for optimal mucosal immune responses during in vivo pulmonary bacterial infection. Proc Natl Acad Sci U S A 2013;110(33):E3119-28.
5. Maizels N. Immunoglobulin gene diversification. Annu Rev Genet 2005;39:23-46.
6. Ahmed R, Bevan MJ, Reiner SL, Fearon DT. The precursors of memory: models and controversies. Nat Rev Immunol 2009;9(9):662-8.
7. Chen K, Kolls JK. T cell-mediated host immune defenses in the lung. Annu Rev Immunol 2013;31:605-33.
8. Marshall NB, Swain SL. Cytotoxic CD4 T cells in antiviral immunity. J Biomed Biotechnol 2011;2011:954602.
9. Mullen AC, High FA, Hutchins AS, Lee HW, Villarino AV, Livingston DM, et al. Role of T-bet in commitment of TH1 cells before IL-12-dependent selection. Science 2001;292(5523):1907-10.
10. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 2000;100(6):655-69.
11. Coffman RL. Origins of the T(H)1-T(H)2 model: a personal perspective. Nat Immunol 2006;7(6):539-41.
12. Murphy KM, Reiner SL. The lineage decisions of helper T cells. Nat Rev Immunol 2002;2(12):933-44.
13. Patient RK, McGhee JD. The GATA family (vertebrates and invertebrates). Curr Opin Genet Dev 2002;12(4):416-22.
14. Sallusto F, Lanzavecchia A. Heterogeneity of CD4+ memory T cells: functional modules for tailored immunity. Eur J Immunol 2009;39(8):2076-82.
15. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? Nat Rev Immunol 2010;10(4):225-35.
16. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol 2005;6(11):1133-41.
17. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005;6(11):1123-32.
18. Manel N, Unutmaz D, Littman DR. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat. Nat Immunol 2008;9(6):641-9.

19. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annu Rev Immunol 2009;27:485-517.

20. Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. N Engl J Med 2009;361(9):888-98.

21. Stockinger B, Veldhoen M. Differentiation and function of Th17 T cells. Curr Opin Immunol 2007;19(3):281-6.

22. Lee Y, Awasthi A, Yosef N, Quintana FJ, Xiao S, Peters A, et al. Induction and molecular signature of pathogenic TH17 cells. Nat Immunol 2012;13(10):991-9.

23. Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, Chung Y, et al. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. Immunity 2008;28(1):29-39.

24. Yang L, Anderson DE, Baecher-Allan C, Hastings WD, Bettelli E, Oukka M, et al. IL-21 and TGF-beta are required for differentiation of human T(H)17 cells. Nature 2008;454(7202):350-2.

25. Mills KH. Induction, function and regulation of IL-17-producing T cells. Eur J Immunol 2008;38(10):2636-49.

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

27. Gu C, Wu L, Li X. IL-17 family: cytokines, receptors and signaling. Cytokine 2013;64(2):477-85.

28. Pappu R, Ramirez-Carrozzi V, Ota N, Ouyang W, Hu Y. The IL-17 family cytokines in immunity and disease. J Clin Immunol 2010;30(2):185-95.

29. Ohkura N, Kitagawa Y, Sakaguchi S. Development and maintenance of regulatory T cells. Immunity 2013;38(3):414-23.

30. Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3+ regulatory T cells in the human immune system. Nat Rev Immunol 2010;10(7):490-500.

31. Samstein RM, Arvey A, Josefowicz SZ, Peng X, Reynolds A, Sandstrom R, et al. Foxp3 exploits a pre-existent enhancer landscape for regulatory T cell lineage specification. Cell 2012;151(1):153-66.

32. McHugh RS, Whitters MJ, Piccirillo CA, Young DA, Shevach EM, Collins M, et al. CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. Immunity 2002;16(2):311-23.

33. Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. J Exp Med 2000;192(2):295-302.

34. Liu W, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, Zhu S, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. J Exp Med 2006;203(7):1701-11.

35. Piccirillo CA. Regulatory T cells in health and disease. Cytokine 2008;43(3):395-401.

36. Gratz IK, Rosenblum MD, Abbas AK. The life of regulatory T cells. Ann N Y Acad Sci 2013;1283:8-12.

37. Abbas AK, Benoist C, Bluestone JA, Campbell DJ, Ghosh S, Hori S, et al. Regulatory T cells: recommendations to simplify the nomenclature. Nat Immunol 2013;14(4):307-8.

38. Thornton AM, Korty PE, Tran DQ, Wohlfert EA, Murray PE, Belkaid Y, et al. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. J Immunol 2010;184(7):3433-41.

39. Himmel ME, MacDonald KG, Garcia RV, Steiner TS, Levings MK. Helios+ and Helios- cells coexist within the natural FOXP3+ T regulatory cell subset in humans. J Immunol 2013;190(5):2001-8.

40. Akimova T, Beier UH, Wang L, Levine MH, Hancock WW. Helios expression is a marker of T cell activation and proliferation. PLoS One 2011;6(8):e24226.

41. Ross EM, Bourges D, Hogan TV, Gleeson PA, van Driel IR. Helios defines T cells being driven to tolerance in the periphery and thymus. Eur J Immunol 2014;44(7):2048-58.

42. Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol 2008;8(7):523-32.

43. Kleinewietfeld M, Hafler DA. Regulatory T cells in autoimmune neuroinflammation. Immunol Rev 2014;259(1):231-44.

44. Shevach EM, Thornton AM. tTregs, pTregs, and iTregs: similarities and differences. Immunol Rev 2014;259(1):88-102.

45. Mottonen M, Heikkinen J, Mustonen L, Isomaki P, Luukkainen R, Lassila O. CD4+ CD25+ T cells with the phenotypic and functional characteristics of regulatory T cells are enriched in the synovial fluid of patients with rheumatoid arthritis. Clin Exp Immunol 2005;140(2):360-7.

46. Nurieva RI, Chung Y, Hwang D, Yang XO, Kang HS, Ma L, et al. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. Immunity 2008;29(1):138-49.

47. Winstead CJ. Follicular helper T cell-mediated mucosal barrier maintenance. Immunol Lett 2014.

48. Crotty S. Follicular helper CD4 T cells (TFH). Annu Rev Immunol 2011;29:621-63.

49. Ma CS, Deenick EK. Human T follicular helper (Tfh) cells and disease. Immunol Cell Biol 2014;92(1):64-71.

50. Schmitt E, Klein M, Bopp T. Th9 cells, new players in adaptive immunity. Trends Immunol 2014;35(2):61-8.

51. Zhao P, Xiao X, Ghobrial RM, Li XC. IL-9 and Th9 cells: progress and challenges. Int Immunol 2013;25(10):547-51.

52. Kaplan MH. Th9 cells: differentiation and disease. Immunol Rev 2013;252(1):104-15.

53. Zhang N, Pan HF, Ye DQ. Th22 in inflammatory and autoimmune disease: prospects for therapeutic intervention. Mol Cell Biochem 2011;353(1-2):41-6.

54. Tian T, Yu S, Ma D. Th22 and related cytokines in inflammatory and autoimmune diseases. Expert Opin Ther Targets 2013;17(2):113-25.

55. Fujita H. The role of IL-22 and Th22 cells in human skin diseases. J Dermatol Sci 2013;72(1):3-8.

56. Swain SL, McKinstry KK, Strutt TM. Expanding roles for CD4(+) T cells in immunity to viruses. Nat Rev Immunol 2012;12(2):136-48.

57. Sakaguchi S. Immunology: Conditional stability of T cells. Nature 2010;468(7320):41-2.

58. O'Shea JJ, Paul WE. Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. Science 2010;327(5969):1098-102.

59. Hori S. Regulatory T cell plasticity: beyond the controversies. Trends Immunol 2011;32(7):295-300.

60. Campbell DJ, Koch MA. Phenotypical and functional specialization of FOXP3+ regulatory T cells. Nat Rev Immunol 2011;11(2):119-30.

61. McClymont SA, Putnam AL, Lee MR, Esensten JH, Liu W, Hulme MA, et al. Plasticity of human regulatory T cells in healthy subjects and patients with type 1 diabetes. J Immunol 2011;186(7):3918-26.

62. Lee YK, Mukasa R, Hatton RD, Weaver CT. Developmental plasticity of Th17 and Treg cells. Curr Opin Immunol 2009;21(3):274-80.

63. Muranski P, Restifo NP. Essentials of Th17 cell commitment and plasticity. Blood 2013;121(13):2402-14.

64. Murphy KM, Stockinger B. Effector T cell plasticity: flexibility in the face of changing circumstances. Nat Immunol 2010;11(8):674-80.

65. Horton R, Wilming L, Rand V, Lovering RC, Bruford EA, Khodiyar VK, et al. Gene map of the extended human MHC. Nat Rev Genet 2004;5(12):889-99.

66. Germain RN. MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. Cell 1994;76(2):287-99.

67. Kaiko GE, Horvat JC, Beagley KW, Hansbro PM. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? Immunology 2008;123(3):326-38.

68. Linsley PS, Nadler SG. The clinical utility of inhibiting CD28-mediated costimulation. Immunol Rev 2009;229(1):307-21.

69. Wang S, Chen L. Co-signaling molecules of the B7-CD28 family in positive and negative regulation of T lymphocyte responses. Microbes Infect 2004;6(8):759-66.

70. Sharpe AH. Mechanisms of costimulation. Immunol Rev 2009;229(1):5-11.

71. Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol Rev 2009;229(1):152-72.

72. Riley JL. PD-1 signaling in primary T cells. Immunol Rev 2009;229(1):114-25.

73. Khoury SJ, Sayegh MH. The roles of the new negative T cell costimulatory pathways in regulating autoimmunity. Immunity 2004;20(5):529-38.

74. Lanzavecchia A, Sallusto F. Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. Science 2000;290(5489):92-7.

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

76. Tam A, Wadsworth S, Dorscheid D, Man SF, Sin DD. The airway epithelium: more than just a structural barrier. Ther Adv Respir Dis 2011;5(4):255-73.

77. Spina D. Epithelium smooth muscle regulation and interactions. Am J Respir Crit Care Med 1998;158(5 Pt 3):S141-5.

78. Jeffery PK. Morphologic features of airway surface epithelial cells and glands. Am Rev Respir Dis 1983;128(2 Pt 2):S14-20.

79. Evans CM, Koo JS. Airway mucus: the good, the bad, the sticky. Pharmacol Ther 2009;121(3):332-48.

80. Knight DA, Holgate ST. The airway epithelium: structural and functional properties in health and disease. Respirology 2003;8(4):432-46.

81. Widdicombe JG, Pack RJ. The Clara cell. Eur J Respir Dis 1982;63(3):202-20.

82. Martin TR, Frevert CW. Innate immunity in the lungs. Proc Am Thorac Soc 2005;2(5):403-11.

83. Nicod LP. Pulmonary defence mechanisms. Respiration 1999;66(1):2-11.

84. Taraseviciene-Stewart L, Voelkel NF. Molecular pathogenesis of emphysema. J Clin Invest 2008;118(2):394-402.

85. Howard TH, Wang D, Berkow RL. Lipopolysaccharide modulates chemotactic peptide-induced actin polymerization in neutrophils. J Leukoc Biol 1990;47(1):13-24.

86. Zarbock A, Ley K. Neutrophil adhesion and activation under flow. Microcirculation 2009;16(1):31-42.

87. Vermaelen KY, Carro-Muino I, Lambrecht BN, Pauwels RA. Specific migratory dendritic cells rapidly transport antigen from the airways to the thoracic lymph nodes. J Exp Med 2001;193(1):51-60.

88. Kallapur SG, Jobe AH. Contribution of inflammation to lung injury and development. Arch Dis Child Fetal Neonatal Ed 2006;91(2):F132-5.

89. King TE, Jr. Clinical advances in the diagnosis and therapy of the interstitial lung diseases. Am J Respir Crit Care Med 2005;172(3):268-79.

90. Antoniou KM, Margaritopoulos GA, Tomassetti S, Bonella F, Costabel U, Poletti V. Interstitial lung disease. Eur Respir Rev 2014;23(131):40-54.

91. DePaso WJ, Winterbauer RH. Interstitial lung disease. Dis Mon 1991;37(2):61-133.

92. Collard HR, King TE, Jr. Demystifying idiopathic interstitial pneumonia. Arch Intern Med 2003;163(1):17-29.

93. Hillerdal G, Nou E, Osterman K, Schmekel B. Sarcoidosis: epidemiology and prognosis. A 15-year European study. Am Rev Respir Dis 1984;130(1):29-32.

94. Baughman RP, Teirstein AS, Judson MA, Rossman MD, Yeager H, Jr., Bresnitz EA, et al. Clinical characteristics of patients in a case control study of sarcoidosis. Am J Respir Crit Care Med 2001;164(10 Pt 1):1885-9.

95. Michielsen HJ, Drent M, Peros-Golubicic T, De Vries J. Fatigue is associated with quality of life in sarcoidosis patients. Chest 2006;130(4):989-94.

96. De Vries J, Drent M. Quality of life and health status in sarcoidosis: a review of the literature. Clin Chest Med 2008;29(3):525-32, ix.

97. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. Am J Respir Crit Care Med 1999;160(2):736-55.

98. Grunewald J. Clinical aspects and immune reactions in sarcoidosis. Clin Respir J 2007;1(2):64-73.

99. Reich JM, Johnson RE. Course and prognosis of sarcoidosis in a nonreferral setting. Analysis of 86 patients observed for 10 years. Am J Med 1985;78(1):61-7.

100. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Engl J Med 2007;357(21):2153-65.

101. Newman KL, Newman LS. Occupational causes of sarcoidosis. Curr Opin Allergy Clin Immunol 2012;12(2):145-50.

102. Saidha S, Sotirchos ES, Eckstein C. Etiology of sarcoidosis: does infection play a role? Yale J Biol Med 2012;85(1):133-41.

103. Bresnitz EA, Strom BL. Epidemiology of sarcoidosis. Epidemiol Rev 1983;5:124-56.

104. Rybicki BA, Amend KL, Maliarik MJ, Iannuzzi MC. Photocopier exposure and risk of sarcoidosis in African-American sibs. Sarcoidosis Vasc Diffuse Lung Dis 2004;21(1):49-55.

105. Newman LS, Rose CS, Bresnitz EA, Rossman MD, Barnard J, Frederick M, et al. A case control etiologic study of sarcoidosis: environmental and occupational risk factors. Am J Respir Crit Care Med 2004;170(12):1324-30.

106. Izbicki G, Chavko R, Banauch GI, Weiden MD, Berger KI, Aldrich TK, et al. World Trade Center "sarcoid-like" granulomatous pulmonary disease in New York City Fire Department rescue workers. Chest 2007;131(5):1414-23.

107. Prezant DJ, Dhala A, Goldstein A, Janus D, Ortiz F, Aldrich TK, et al. The incidence, prevalence, and severity of sarcoidosis in New York City firefighters. Chest 1999;116(5):1183-93.

108. Saboor SA, Johnson NM, McFadden J. Detection of mycobacterial DNA in sarcoidosis and tuberculosis with polymerase chain reaction. Lancet 1992;339(8800):1012-5.

109. Eishi Y, Suga M, Ishige I, Kobayashi D, Yamada T, Takemura T, et al. Quantitative analysis of mycobacterial and propionibacterial DNA in lymph nodes of Japanese and European patients with sarcoidosis. J Clin Microbiol 2002;40(1):198-204.

110. Gupta D, Agarwal R, Aggarwal AN, Jindal SK. Molecular evidence for the role of mycobacteria in sarcoidosis: a meta-analysis. Eur Respir J 2007;30(3):508-16.

111. Chen ES, Moller DR. Etiologic role of infectious agents. Semin Respir Crit Care Med 2014;35(3):285-95.

112. Brownell I, Ramirez-Valle F, Sanchez M, Prystowsky S. Evidence for mycobacteria in sarcoidosis. Am J Respir Cell Mol Biol 2011;45(5):899-905.

113. Hajizadeh R, Sato H, Carlisle J, Nadaf MT, Evans W, Shepherd BE, et al. Mycobacterium tuberculosis Antigen 85A induces Th-1 immune responses in systemic sarcoidosis. J Clin Immunol 2007;27(4):445-54.

114. Dubaniewicz A, Trzonkowski P, Dubaniewicz-Wybieralska M, Singh M, Mysliwski A. Mycobacterial heat shock protein-induced blood T lymphocytes subsets and cytokine pattern: comparison of sarcoidosis with tuberculosis and healthy controls. Respirology 2007;12(3):346-54.

115. Oswald-Richter KA, Beachboard DC, Seeley EH, Abraham S, Shepherd BE, Jenkins CA, et al. Dual analysis for mycobacteria and propionibacteria in sarcoidosis BAL. J Clin Immunol 2012;32(5):1129-40.

116. Teirstein AS. Kveim antigen: what does it tell us about causation of sarcoidosis? Semin Respir Infect 1998;13(3):206-11.

117. Song Z, Marzilli L, Greenlee BM, Chen ES, Silver RF, Askin FB, et al. Mycobacterial catalase-peroxidase is a tissue antigen and target of the adaptive immune response in systemic sarcoidosis. J Exp Med 2005;201(5):755-67.

118. Oswald-Richter KA, Culver DA, Hawkins C, Hajizadeh R, Abraham S, Shepherd BE, et al. Cellular responses to mycobacterial antigens are present in bronchoalveolar lavage fluid used in the diagnosis of sarcoidosis. Infect Immun 2009;77(9):3740-8.

119. Drake WP, Dhason MS, Nadaf M, Shepherd BE, Vadivelu S, Hajizadeh R, et al. Cellular recognition of Mycobacterium tuberculosis ESAT-6 and KatG peptides in systemic sarcoidosis. Infect Immun 2007;75(1):527-30.

120. Chen ES, Wahlstrom J, Song Z, Willett MH, Wiken M, Yung RC, et al. T cell responses to mycobacterial catalase-peroxidase profile a pathogenic antigen in systemic sarcoidosis. J Immunol 2008;181(12):8784-96.

121. Agostini C, Semenzato G, James DG. Immunological, clinical and molecular aspects of sarcoidosis. Mol Aspects Med 1997;18(2):91-165.

122. Zissel G, Prasse A, Muller-Quernheim J. Immunologic response of sarcoidosis. Semin Respir Crit Care Med 2010;31(4):390-403.

123. Muller-Quernheim J, Prasse A, Zissel G. Pathogenesis of sarcoidosis. Presse Med 2012;41(6 Pt 2):e275-87.

124. Welker L, Jorres RA, Costabel U, Magnussen H. Predictive value of BAL cell differentials in the diagnosis of interstitial lung diseases. Eur Respir J 2004;24(6):1000-6.

125. Facco M, Cabrelle A, Teramo A, Olivieri V, Gnoato M, Teolato S, et al. Sarcoidosis is a Th1/Th17 multisystem disorder. Thorax 2011;66(2):144-50.

126. Ten Berge B, Paats MS, Bergen IM, van den Blink B, Hoogsteden HC, Lambrecht BN, et al. Increased IL-17A expression in granulomas and in circulating memory T cells in sarcoidosis. Rheumatology (Oxford) 2012;51(1):37-46.

127. Chen ES, Moller DR. Sarcoidosis--scientific progress and clinical challenges. Nat Rev Rheumatol 2011;7(8):457-67.

128. Moller DR, Forman JD, Liu MC, Noble PW, Greenlee BM, Vyas P, et al. Enhanced expression of IL-12 associated with Th1 cytokine profiles in active pulmonary sarcoidosis. J Immunol 1996;156(12):4952-60.

129. Walker C, Bauer W, Braun RK, Menz G, Braun P, Schwarz F, et al. Activated T cells and cytokines in bronchoalveolar lavages from patients with various lung diseases associated with eosinophilia. Am J Respir Crit Care Med 1994;150(4):1038-48.

130. Greene CM, Meachery G, Taggart CC, Rooney CP, Coakley R, O'Neill SJ, et al. Role of IL-18 in CD4+ T lymphocyte activation in sarcoidosis. J Immunol 2000;165(8):4718-24.

131. Larousserie F, Pflanz S, Coulomb-L'Hermine A, Brousse N, Kastelein R, Devergne O. Expression of IL-27 in human Th1-associated granulomatous diseases. J Pathol 2004;202(2):164-71.

132. Katchar K, Eklund A, Grunewald J. Expression of Th1 markers by lung accumulated T cells in pulmonary sarcoidosis. J Intern Med 2003;254(6):564-71.

133. Broos CE, van Nimwegen M, Hoogsteden HC, Hendriks RW, Kool M, van den Blink B. Granuloma formation in pulmonary sarcoidosis. Front Immunol 2013;4:437.

134. Valeyre D, Prasse A, Nunes H, Uzunhan Y, Brillet PY, Muller-Quernheim J. Sarcoidosis. Lancet 2014;383(9923):1155-67.

135. Bradley B, Branley HM, Egan JJ, Greaves MS, Hansell DM, Harrison NK, et al. Interstitial lung disease guideline: the British Thoracic Society in collaboration with the Thoracic Society of Australia and New Zealand and the Irish Thoracic Society. Thorax 2008;63 Suppl 5:v1-58.

136. Hunninghake GW, Costabel U, Ando M, Baughman R, Cordier JF, du Bois R, et al. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. Sarcoidosis Vasc Diffuse Lung Dis 1999;16(2):149-73.

137. Baughman RP, Lower EE, du Bois RM. Sarcoidosis. Lancet 2003;361(9363):1111-8.

138. Martinet Y, Pinkston P, Saltini C, Spurzem J, Muller-Quernheim J, Crystal RG. Evaluation of the in vitro and in vivo effects of cyclosporine on the lung T-lymphocyte alveolitis of active pulmonary sarcoidosis. Am Rev Respir Dis 1988;138(5):1242-8.

139. Elfferich MD, Nelemans PJ, Ponds RW, De Vries J, Wijnen PA, Drent M. Everyday cognitive failure in sarcoidosis: the prevalence and the effect of anti-TNF-alpha treatment. Respiration 2010;80(3):212-9.

140. Grunewald J, Eklund A. Lofgren's syndrome: human leukocyte antigen strongly influences the disease course. Am J Respir Crit Care Med 2009;179(4):307-12.

141. Berlin M, Fogdell-Hahn A, Olerup O, Eklund A, Grunewald J. HLA-DR predicts the prognosis in Scandinavian patients with pulmonary sarcoidosis. Am J Respir Crit Care Med 1997;156(5):1601-5.

142. Grunewald J, Eklund A, Olerup O. Human leukocyte antigen class I alleles and the disease course in sarcoidosis patients. Am J Respir Crit Care Med 2004;169(6):696-702.

143. Grunewald J, Janson CH, Eklund A, Ohrn M, Olerup O, Persson U, et al. Restricted V alpha 2.3 gene usage by CD4+ T lymphocytes in bronchoalveolar lavage fluid from sarcoidosis patients correlates with HLA-DR3. Eur J Immunol 1992;22(1):129-35.

144. Grunewald J, Olerup O, Persson U, Ohrn MB, Wigzell H, Eklund A. T-cell receptor variable region gene usage by CD4+ and CD8+ T cells in bronchoalveolar lavage fluid and peripheral blood of sarcoidosis patients. Proc Natl Acad Sci U S A 1994;91(11):4965-9.

145. Wahlstrom J, Dengjel J, Winqvist O, Targoff I, Persson B, Duyar H, et al. Autoimmune T cell responses to antigenic peptides presented by bronchoalveolar lavage cell HLA-DR molecules in sarcoidosis. Clin Immunol 2009;133(3):353-63.

146. Ahlgren KM, Ruckdeschel T, Eklund A, Wahlstrom J, Grunewald J. T cell receptor-Vbeta repertoires in lung and blood CD4+ and CD8+ T cells of pulmonary sarcoidosis patients. BMC Pulm Med 2014;14:50.

147. Chen ES, Song Z, Willett MH, Heine S, Yung RC, Liu MC, et al. Serum amyloid A regulates granulomatous inflammation in sarcoidosis through Toll-like receptor-2. Am J Respir Crit Care Med 2010;181(4):360-73.

148. Jha P, Ranson MK, Nguyen SN, Yach D. Estimates of global and regional smoking prevalence in 1995, by age and sex. Am J Public Health 2002;92(6):1002-6.

149. Stedman RL. The chemical composition of tobacco and tobacco smoke. Chem Rev 1968;68(2):153-207.

150. Talhout R, Schulz T, Florek E, van Benthem J, Wester P, Opperhuizen A. Hazardous compounds in tobacco smoke. Int J Environ Res Public Health 2011;8(2):613-28.

151. Arnson Y, Shoenfeld Y, Amital H. Effects of tobacco smoke on immunity, inflammation and autoimmunity. J Autoimmun 2010;34(3):J258-65.

152. Yang SR, Chida AS, Bauter MR, Shafiq N, Seweryniak K, Maggirwar SB, et al. Cigarette smoke induces proinflammatory cytokine release by activation of NF-kappaB and posttranslational modifications of histone deacetylase in macrophages. Am J Physiol Lung Cell Mol Physiol 2006;291(1):L46-57.

153. Bermudez EA, Rifai N, Buring JE, Manson JE, Ridker PM. Relation between markers of systemic vascular inflammation and smoking in women. Am J Cardiol 2002;89(9):1117-9.

154. Glossop JR, Dawes PT, Mattey DL. Association between cigarette smoking and release of tumour necrosis factor alpha and its soluble receptors by peripheral blood mononuclear cells in patients with rheumatoid arthritis. Rheumatology (Oxford) 2006;45(10):1223-9.

155. Hagiwara E, Takahashi KI, Okubo T, Ohno S, Ueda A, Aoki A, et al. Cigarette smoking depletes cells spontaneously secreting Th(1) cytokines in the human airway. Cytokine 2001;14(2):121-6.

156. Lindblad SS, Mydel P, Jonsson IM, Senior RM, Tarkowski A, Bokarewa M. Smoking and nicotine exposure delay development of collagen-induced arthritis in mice. Arthritis Res Ther 2009;11(3):R88.

157. Nizri E, Irony-Tur-Sinai M, Lory O, Orr-Urtreger A, Lavi E, Brenner T. Activation of the cholinergic anti-inflammatory system by nicotine attenuates neuroinflammation via suppression of Th1 and Th17 responses. J Immunol 2009;183(10):6681-8.

158. Lee J, Taneja V, Vassallo R. Cigarette smoking and inflammation: cellular and molecular mechanisms. J Dent Res 2012;91(2):142-9.

159. Modestou MA, Manzel LJ, El-Mahdy S, Look DC. Inhibition of IFN-gamma-dependent antiviral airway epithelial defense by cigarette smoke. Respir Res 2010;11:64.

160. Hutchinson D, Shepstone L, Moots R, Lear JT, Lynch MP. Heavy cigarette smoking is strongly associated with rheumatoid arthritis (RA), particularly in patients without a family history of RA. Ann Rheum Dis 2001;60(3):223-7.

161. van der Heide F, Dijkstra A, Weersma RK, Albersnagel FA, van der Logt EM, Faber KN, et al. Effects of active and passive smoking on disease course of Crohn's disease and ulcerative colitis. Inflamm Bowel Dis 2009;15(8):1199-207.

162. Klareskog L, Stolt P, Lundberg K, Kallberg H, Bengtsson C, Grunewald J, et al. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum 2006;54(1):38-46.

163. Makrygiannakis D, Hermansson M, Ulfgren AK, Nicholas AP, Zendman AJ, Eklund A, et al. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. Ann Rheum Dis 2008;67(10):1488-92.

164. Ekblom-Kullberg S, Kautiainen H, Alha P, Leirisalo-Repo M, Miettinen A, Julkunen H. Smoking, disease activity, permanent damage and dsDNA autoantibody production in patients with systemic lupus erythematosus. Rheumatol Int 2014;34(3):341-5.

165. Lassmann H, Raine CS, Antel J, Prineas JW. Immunopathology of multiple sclerosis: report on an international meeting held at the Institute of Neurology of the University of Vienna. J Neuroimmunol 1998;86(2):213-7.

166. Severson C, Hafler DA. T-cells in multiple sclerosis. Results Probl Cell Differ 2010;51:75-98.

167. Kamm CP, Uitdehaag BM, Polman CH. Multiple sclerosis: current knowledge and future outlook. Eur Neurol 2014;72(3-4):132-41.

168. Hafler DA. Multiple sclerosis. J Clin Invest 2004;113(6):788-94.

169. Prineas J. Pathology of the early lesion in multiple sclerosis. Hum Pathol 1975;6(5):531-54.

170. Zhang J, Markovic-Plese S, Lacet B, Raus J, Weiner HL, Hafler DA. Increased frequency of interleukin 2-responsive T cells specific for myelin basic protein and proteolipid protein in peripheral blood and cerebrospinal fluid of patients with multiple sclerosis. J Exp Med 1994;179(3):973-84.

171. Hedstrom AK, Sundqvist E, Baarnhielm M, Nordin N, Hillert J, Kockum I, et al. Smoking and two human leukocyte antigen genes interact to increase the risk for multiple sclerosis. Brain 2011;134(Pt 3):653-64.

172. Zivadinov R, Weinstock-Guttman B, Hashmi K, Abdelrahman N, Stosic M, Dwyer M, et al. Smoking is associated with increased lesion volumes and brain atrophy in multiple sclerosis. Neurology 2009;73(7):504-10.

173. Nylander A, Hafler DA. Multiple sclerosis. J Clin Invest 2012;122(4):1180-8.

174. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011;69(2):292-302.

175. Eklund A, Blaschke E. Relationship between changed alveolar-capillary permeability and angiotensin converting enzyme activity in serum in sarcoidosis. Thorax 1986;41(8):629-34.

176. Zhang Y, Heym B, Allen B, Young D, Cole S. The catalase-peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. Nature 1992;358(6387):591-3.

177. Borsuk S, Newcombe J, Mendum TA, Dellagostin OA, McFadden J. Identification of proteins from tuberculin purified protein derivative (PPD) by LC-MS/MS. Tuberculosis (Edinb) 2009;89(6):423-30.

178. White J, Herman A, Pullen AM, Kubo R, Kappler JW, Marrack P. The V beta-specific superantigen staphylococcal enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice. Cell 1989;56(1):27-35.

179. Winterbauer RH, Lammert J, Selland M, Wu R, Corley D, Springmeyer SC. Bronchoalveolar lavage cell populations in the diagnosis of sarcoidosis. Chest 1993;104(2):352-61.

180. Planck A, Eklund A, Grunewald J. Markers of activity in clinically recovered human leukocyte antigen-DR17-positive sarcoidosis patients. Eur Respir J 2003;21(1):52-7.

181. Darrah PA, Patel DT, De Luca PM, Lindsay RW, Davey DF, Flynn BJ, et al. Multifunctional TH1 cells define a correlate of vaccine-mediated protection against Leishmania major. Nat Med 2007;13(7):843-50.

182. Glader P, Smith ME, Malmhall C, Balder B, Sjostrand M, Qvarfordt I, et al. Interleukin-17-producing T-helper cells and related cytokines in human airways exposed to endotoxin. Eur Respir J 2010;36(5):1155-64.

183. Niemeyer CM, Adler M, Wacker R. Detecting antigens by quantitative immuno-PCR. Nat Protoc 2007;2(8):1918-30.

184. Judson MA. The Clinical Features of Sarcoidosis: A Comprehensive Review. Clin Rev Allergy Immunol 2014.

185. Wahlstrom J, Katchar K, Wigzell H, Olerup O, Eklund A, Grunewald J. Analysis of intracellular cytokines in CD4+ and CD8+ lung and blood T cells in sarcoidosis. Am J Respir Crit Care Med 2001;163(1):115-21.

186. Pinkston P, Bitterman PB, Crystal RG. Spontaneous release of interleukin-2 by lung T lymphocytes in active pulmonary sarcoidosis. N Engl J Med 1983;308(14):793-800.

187. Minshall EM, Tsicopoulos A, Yasruel Z, Wallaert B, Akoum H, Vorng H, et al. Cytokine mRNA gene expression in active and nonactive pulmonary sarcoidosis. Eur Respir J 1997;10(9):2034-9.

188. Muller-Quernheim J, Prasse A, Moller DR. "Sarcoidosis: recent advances". Semin Respir Crit Care Med 2014;35(3):283-4.

189. Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. Disseminated tuberculosis in interferon gamma gene-disrupted mice. J Exp Med 1993;178(6):2243-7.

190. Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon gamma in resistance to Mycobacterium tuberculosis infection. J Exp Med 1993;178(6):2249-54.

191. Bloom BR, Flynn J, McDonough K, Kress Y, Chan J. Experimental approaches to mechanisms of protection and pathogenesis in M. tuberculosis infection. Immunobiology 1994;191(4-5):526-36.

192. Boussiotis VA, Barber DL, Nakarai T, Freeman GJ, Gribben JG, Bernstein GM, et al. Prevention of T cell anergy by signaling through the gamma c chain of the IL-2 receptor. Science 1994;266(5187):1039-42.

193. Orme IM, Collins FM. Protection against Mycobacterium tuberculosis infection by adoptive immunotherapy. Requirement for T cell-deficient recipients. J Exp Med 1983;158(1):74-83.

194. Rojas RE, Balaji KN, Subramanian A, Boom WH. Regulation of human CD4(+) alphabeta T-cell-receptor-positive (TCR(+)) and gammadelta TCR(+) T-cell responses to Mycobacterium tuberculosis by interleukin-10 and transforming growth factor beta. Infect Immun 1999;67(12):6461-72.

195. Chackerian AA, Perera TV, Behar SM. Gamma interferon-producing CD4+ T lymphocytes in the lung correlate with resistance to infection with Mycobacterium tuberculosis. Infect Immun 2001;69(4):2666-74.

196. MacMicking JD, North RJ, LaCourse R, Mudgett JS, Shah SK, Nathan CF. Identification of nitric oxide synthase as a protective locus against tuberculosis. Proc Natl Acad Sci U S A 1997;94(10):5243-8.

197. Flynn JL, Chan J. Immunology of tuberculosis. Annu Rev Immunol 2001;19:93-129.

198. Sousa AO, Mazzaccaro RJ, Russell RG, Lee FK, Turner OC, Hong S, et al. Relative contributions of distinct MHC class I-dependent cell populations in protection to tuberculosis infection in mice. Proc Natl Acad Sci U S A 2000;97(8):4204-8.

199. Feng CG, Bean AG, Hooi H, Briscoe H, Britton WJ. Increase in gamma interferon-secreting CD8(+), as well as CD4(+), T cells in lungs following aerosol infection with Mycobacterium tuberculosis. Infect Immun 1999;67(7):3242-7.

200. Serbina NV, Liu CC, Scanga CA, Flynn JL. CD8+ CTL from lungs of Mycobacterium tuberculosis-infected mice express perforin in vivo and lyse infected macrophages. J Immunol 2000;165(1):353-63.

201. Mittrucker HW, Steinhoff U, Kohler A, Krause M, Lazar D, Mex P, et al. Poor correlation between BCG vaccination-induced T cell responses and protection against tuberculosis. Proc Natl Acad Sci U S A 2007;104(30):12434-9.

202. Gonzalez-Juarrero M, Turner OC, Turner J, Marietta P, Brooks JV, Orme IM. Temporal and spatial arrangement of lymphocytes within lung granulomas induced by aerosol infection with Mycobacterium tuberculosis. Infect Immun 2001;69(3):1722-8.

203. North RJ, Jung YJ. Immunity to tuberculosis. Annu Rev Immunol 2004;22:599-623.

204. Muller-Quernheim J, Saltini C, Sondermeyer P, Crystal RG. Compartmentalized activation of the interleukin 2 gene by lung T lymphocytes in active pulmonary sarcoidosis. J Immunol 1986;137(11):3475-83.

205. Oswald-Richter KA, Beachboard DC, Zhan X, Gaskill CF, Abraham S, Jenkins C, et al. Multiple mycobacterial antigens are targets of the adaptive immune response in pulmonary sarcoidosis. Respir Res 2010;11:161.

206. Carlisle J, Evans W, Hajizadeh R, Nadaf M, Shepherd B, Ott RD, et al. Multiple Mycobacterium antigens induce interferon-gamma production from sarcoidosis peripheral blood mononuclear cells. Clin Exp Immunol 2007;150(3):460-8.

207. Blackwell JM. Genetic susceptibility to leishmanial infections: studies in mice and man. Parasitology 1996;112 Suppl:S67-74.

208. Bogdan C, Moll H, Solbach W, Rollinghoff M. Tumor necrosis factor-alpha in combination with interferon-gamma, but not with interleukin 4 activates murine macrophages for elimination of Leishmania major amastigotes. Eur J Immunol 1990;20(5):1131-5.

209. Liew FY, Li Y, Millott S. Tumor necrosis factor-alpha synergizes with IFN-gamma in mediating killing of Leishmania major through the induction of nitric oxide. J Immunol 1990;145(12):4306-10.

210. Forbes EK, Sander C, Ronan EO, McShane H, Hill AV, Beverley PC, et al. Multifunctional, high-level cytokine-producing Th1 cells in the lung, but not spleen, correlate with protection against Mycobacterium tuberculosis aerosol challenge in mice. J Immunol 2008;181(7):4955-64.

211. Idali F, Wiken M, Wahlstrom J, Mellstedt H, Eklund A, Rabbani H, et al. Reduced Th1 response in the lungs of HLA-DRB1*0301 patients with pulmonary sarcoidosis. Eur Respir J 2006;27(3):451-9.

212. Krutzik SR, Modlin RL. The role of Toll-like receptors in combating mycobacteria. Semin Immunol 2004;16(1):35-41.

213. Seder RA, Darrah PA, Roederer M. T-cell quality in memory and protection: implications for vaccine design. Nat Rev Immunol 2008;8(4):247-58.

214. Wang ZE, Reiner SL, Zheng S, Dalton DK, Locksley RM. CD4+ effector cells default to the Th2 pathway in interferon gamma-deficient mice infected with Leishmania major. J Exp Med 1994;179(4):1367-71.

215. Skinner MA, Ramsay AJ, Buchan GS, Keen DL, Ranasinghe C, Slobbe L, et al. A DNA prime-live vaccine boost strategy in mice can augment IFN-gamma responses to mycobacterial antigens but does not increase the protective efficacy of two attenuated strains of Mycobacterium bovis against bovine tuberculosis. Immunology 2003;108(4):548-55.

216. Elias D, Akuffo H, Britton S. PPD induced in vitro interferon gamma production is not a reliable correlate of protection against Mycobacterium tuberculosis. Trans R Soc Trop Med Hyg 2005;99(5):363-8.

217. Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, et al. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. Lancet 1999;354(9194):1932-9.

218. Chan J, Xing Y, Magliozzo RS, Bloom BR. Killing of virulent Mycobacterium tuberculosis by reactive nitrogen intermediates produced by activated murine macrophages. J Exp Med 1992;175(4):1111-22.

219. Winkler S, Necek M, Winkler H, Adegnika AA, Perkmann T, Ramharter M, et al. Increased specific T cell cytokine responses in patients with active pulmonary tuberculosis from Central Africa. Microbes Infect 2005;7(9-10):1161-9.

220. Soares AP, Scriba TJ, Joseph S, Harbacheuski R, Murray RA, Gelderbloem SJ, et al. Bacillus Calmette-Guerin vaccination of human newborns induces T cells with complex cytokine and phenotypic profiles. J Immunol 2008;180(5):3569-77.

221. Scriba TJ, Kalsdorf B, Abrahams DA, Isaacs F, Hofmeister J, Black G, et al. Distinct, specific IL-17- and IL-22-producing CD4+ T cell subsets contribute to the human anti-mycobacterial immune response. J Immunol 2008;180(3):1962-70.

222. Lindenstrom T, Agger EM, Korsholm KS, Darrah PA, Aagaard C, Seder RA, et al. Tuberculosis subunit vaccination provides long-term protective immunity characterized by multifunctional CD4 memory T cells. J Immunol 2009;182(12):8047-55.

223. Huaman MC, Mullen GE, Long CA, Mahanty S. Plasmodium falciparum apical membrane antigen 1 vaccine elicits multifunctional CD4 cytokine-producing and memory T cells. Vaccine 2009;27(38):5239-46.

224. Goonetilleke N, Moore S, Dally L, Winstone N, Cebere I, Mahmoud A, et al. Induction of multifunctional human immunodeficiency virus type 1 (HIV-1)-specific T cells capable of proliferation in healthy subjects by using a prime-boost regimen of DNA- and modified vaccinia virus Ankara-vectored vaccines expressing HIV-1 Gag coupled to CD8+ T-cell epitopes. J Virol 2006;80(10):4717-28.

225. Esin S, Batoni G, Saruhan-Direskeneli G, Harris RA, Grunewald J, Pardini M, et al. In vitro expansion of T-cell-receptor Valpha2.3(+) CD4(+) T lymphocytes in HLA-DR17(3), DQ2(+) individuals upon stimulation with Mycobacterium tuberculosis. Infect Immun 1999;67(8):3800-9.

226. Lehmann C, Wilkening A, Leiber D, Markus A, Krug N, Pabst R, et al. Lymphocytes in the bronchoalveolar space reenter the lung tissue by means of the alveolar epithelium, migrate to regional lymph nodes, and subsequently rejoin the systemic immune system. Anat Rec 2001;264(3):229-36.

227. Grunewald J, Berlin M, Olerup O, Eklund A. Lung T-helper cells expressing T-cell receptor AV2S3 associate with clinical features of pulmonary sarcoidosis. Am J Respir Crit Care Med 2000;161(3 Pt 1):814-8.

228. Katchar K, Wahlstrom J, Eklund A, Grunewald J. Highly activated T-cell receptor AV2S3(+) CD4(+) lung T-cell expansions in pulmonary sarcoidosis. Am J Respir Crit Care Med 2001;163(7):1540-5.

229. Wiken M, Grunewald J, Eklund A, Wahlstrom J. Multiparameter phenotyping of T-cell subsets in distinct subgroups of patients with pulmonary sarcoidosis. J Intern Med 2012;271(1):90-103.

230. Idali F, Wahlstrom J, Muller-Suur C, Eklund A, Grunewald J. Analysis of regulatory T cell associated forkhead box P3 expression in the lungs of patients with sarcoidosis. Clin Exp Immunol 2008;152(1):127-37.

231. Grunewald J, Hultman T, Bucht A, Eklund A, Wigzell H. Restricted usage of T cell receptor V alpha/J alpha gene segments with different nucleotide but identical amino acid sequences in HLA-DR3+ sarcoidosis patients. Mol Med 1995;1(3):287-96.

232. Mallone R, Kochik SA, Laughlin EM, Gersuk VH, Reijonen H, Kwok WW, et al. Differential recognition and activation thresholds in human autoreactive GAD-specific T-cells. Diabetes 2004;53(4):971-7.

233. Mack DG, Lanham AM, Palmer BE, Maier LA, Fontenot AP. CD27 expression on CD4+ T cells differentiates effector from regulatory T cell subsets in the lung. J Immunol 2009;182(11):7317-24.

234. van Lier RA, Borst J, Vroom TM, Klein H, Van Mourik P, Zeijlemaker WP, et al. Tissue distribution and biochemical and functional properties of Tp55 (CD27), a novel T cell differentiation antigen. J Immunol 1987;139(5):1589-96.

235. Hamann D, Baars PA, Rep MH, Hooibrink B, Kerkhof-Garde SR, Klein MR, et al. Phenotypic and functional separation of memory and effector human CD8+ T cells. J Exp Med 1997;186(9):1407-18.

236. Hintzen RQ, de Jong R, Lens SM, Brouwer M, Baars P, van Lier RA. Regulation of CD27 expression on subsets of mature T-lymphocytes. J Immunol 1993;151(5):2426-35.

237. Wynn TA. T(H)-17: a giant step from T(H)1 and T(H)2. Nat Immunol 2005;6(11):1069-70.

238. Kolls JK. CD4(+) T-cell subsets and host defense in the lung. Immunol Rev 2013;252(1):156-63.

239. Ye P, Garvey PB, Zhang P, Nelson S, Bagby G, Summer WR, et al. Interleukin-17 and lung host defense against Klebsiella pneumoniae infection. Am J Respir Cell Mol Biol 2001;25(3):335-40.

240. Trautwein-Weidner K, Gladiator A, Nur S, Diethelm P, LeibundGut-Landmann S. IL-17-mediated antifungal defense in the oral mucosa is independent of neutrophils. Mucosal Immunol 2014.

241. Curtis MM, Way SS. Interleukin-17 in host defence against bacterial, mycobacterial and fungal pathogens. Immunology 2009;126(2):177-85.

242. Torchinsky MB, Blander JM. T helper 17 cells: discovery, function, and physiological trigger. Cell Mol Life Sci 2010;67(9):1407-21.

243. Klareskog L, Catrina AI, Paget S. Rheumatoid arthritis. Lancet 2009;373(9664):659-72.

244. Okamoto Yoshida Y, Umemura M, Yahagi A, O'Brien RL, Ikuta K, Kishihara K, et al. Essential role of IL-17A in the formation of a mycobacterial infection-induced granuloma in the lung. J Immunol 2010;184(8):4414-22.

245. Vanaudenaerde BM, Verleden SE, Vos R, De Vleeschauwer SI, Willems-Widyastuti A, Geenens R, et al. Innate and adaptive interleukin-17-producing lymphocytes in chronic inflammatory lung disorders. Am J Respir Crit Care Med 2011;183(8):977-86.

246. Hashimoto T, Akiyama K, Kobayashi N, Mori A. Comparison of IL-17 production by helper T cells among atopic and nonatopic asthmatics and control subjects. Int Arch Allergy Immunol 2005;137 Suppl 1:51-4.

247. Nembrini C, Marsland BJ, Kopf M. IL-17-producing T cells in lung immunity and inflammation. J Allergy Clin Immunol 2009;123(5):986-94; quiz 995-6.

248. Urbankowski T, Hoser G, Domagala-Kulawik J. Th1/Th2/Th17related cytokines in the bronchoalveolar lavage fluid of patients with sarcoidosis: association with smoking. Pol Arch Med Wewn 2012;122(7-8):320-5.

249. Furusawa H, Suzuki Y, Miyazaki Y, Inase N, Eishi Y. Th1 and Th17 immune responses to viable Propionibacterium acnes in patients with sarcoidosis. Respir Investig 2012;50(3):104-9.

250. Richmond BW, Ploetze K, Isom J, Chambers-Harris I, Braun NA, Taylor T, et al. Sarcoidosis Th17 cells are ESAT-6 antigen specific but demonstrate reduced IFN-gamma expression. J Clin Immunol 2013;33(2):446-55.

251. Tondell A, Moen T, Borset M, Salvesen O, Ro AD, Sue-Chu M. Bronchoalveolar lavage fluid IFN-gamma+ Th17 cells and regulatory T cells in pulmonary sarcoidosis. Mediators Inflamm 2014;2014:438070.

252. Wiken M, Ostadkarampour M, Eklund A, Willett M, Chen E, Moller D, et al. Antigen-specific multifunctional T-cells in sarcoidosis patients with Lofgren's syndrome. Eur Respir J 2012;40(1):110-21.

253. Singh RP, Hasan S, Sharma S, Nagra S, Yamaguchi DT, Wong D, et al. Th17 cells in inflammation and autoimmunity. Autoimmun Rev 2014.

254. Khader SA, Bell GK, Pearl JE, Fountain JJ, Rangel-Moreno J, Cilley GE, et al. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during Mycobacterium tuberculosis challenge. Nat Immunol 2007;8(4):369-77.

255. Yao Z, Fanslow WC, Seldin MF, Rousseau AM, Painter SL, Comeau MR, et al. Herpesvirus Saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. Immunity 1995;3(6):811-21.

256. Umemura M, Yahagi A, Hamada S, Begum MD, Watanabe H, Kawakami K, et al. IL-17-mediated regulation of innate and acquired immune response against pulmonary Mycobacterium bovis bacille Calmette-Guerin infection. J Immunol 2007;178(6):3786-96.

257. Wilke CM, Bishop K, Fox D, Zou W. Deciphering the role of Th17 cells in human disease. Trends Immunol 2011;32(12):603-11.

258. Maddur MS, Miossec P, Kaveri SV, Bayry J. Th17 cells: biology, pathogenesis of autoimmune and inflammatory diseases, and therapeutic strategies. Am J Pathol 2012;181(1):8-18.

259. Linden A. Role of interleukin-17 and the neutrophil in asthma. Int Arch Allergy Immunol 2001;126(3):179-84.

260. Sergejeva S, Ivanov S, Lotvall J, Linden A. Interleukin-17 as a recruitment and survival factor for airway macrophages in allergic airway inflammation. Am J Respir Cell Mol Biol 2005;33(3):248-53.

261. Peng Y, Han G, Shao H, Wang Y, Kaplan HJ, Sun D. Characterization of IL-17+ interphotoreceptor retinoid-binding protein-specific T cells in experimental autoimmune uveitis. Invest Ophthalmol Vis Sci 2007;48(9):4153-61.

262. Ke Y, Liu K, Huang GQ, Cui Y, Kaplan HJ, Shao H, et al. Anti-inflammatory role of IL-17 in experimental autoimmune uveitis. J Immunol 2009;182(5):3183-90.

263. O'Connor W, Jr., Kamanaka M, Booth CJ, Town T, Nakae S, Iwakura Y, et al. A protective function for interleukin 17A in T cell-mediated intestinal inflammation. Nat Immunol 2009;10(6):603-9.

264. Monteleone I, Sarra M, Pallone F, Monteleone G. Th17-related cytokines in inflammatory bowel diseases: friends or foes? Curr Mol Med 2012;12(5):592-7.

265. Lee YK, Turner H, Maynard CL, Oliver JR, Chen D, Elson CO, et al. Late developmental plasticity in the T helper 17 lineage. Immunity 2009;30(1):92-107.

266. Ghoreschi K, Laurence A, Yang XP, Tato CM, McGeachy MJ, Konkel JE, et al. Generation of pathogenic T(H)17 cells in the absence of TGF-beta signalling. Nature 2010;467(7318):967-71.

267. Genovese MC, Van den Bosch F, Roberson SA, Bojin S, Biagini IM, Ryan P, et al. LY2439821, a humanized anti-interleukin-17 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: A phase I randomized, double-blind, placebo-controlled, proof-of-concept study. Arthritis Rheum 2010;62(4):929-39.

268. Waisman A. To be 17 again--anti-interleukin-17 treatment for psoriasis. N Engl J Med 2012;366(13):1251-2.

269. Forsslund H, Mikko M, Karimi R, Grunewald J, Wheelock AM, Wahlstrom J, et al. Distribution of T-cell subsets in BAL fluid of patients with mild to moderate COPD

depends on current smoking status and not airway obstruction. Chest 2014;145(4):711-22.

270. Barnes PJ, Cosio MG. Characterization of T lymphocytes in chronic obstructive pulmonary disease. PLoS Med 2004;1(1):e20.

271. Sdralia ND, Patmanidi AL, Velentzas AD, Margaritis LH, Baltatzis GE, Hatzinikolaou DG, et al. The mode of lymphoblastoid cell death in response to gas phase cigarette smoke is dose-dependent. Respir Res 2009;10:82.

272. Qureshi H, Sharafkhaneh A, Hanania NA. Chronic obstructive pulmonary disease exacerbations: latest evidence and clinical implications. Ther Adv Chronic Dis 2014;5(5):212-27.

273. Caramori G, Adcock IM, Di Stefano A, Chung KF. Cytokine inhibition in the treatment of COPD. Int J Chron Obstruct Pulmon Dis 2014;9:397-412.

274. Di Stefano A, Caramori G, Gnemmi I, Contoli M, Vicari C, Capelli A, et al. T helper type 17-related cytokine expression is increased in the bronchial mucosa of stable chronic obstructive pulmonary disease patients. Clin Exp Immunol 2009;157(2):316-24.

275. Chang Y, Nadigel J, Boulais N, Bourbeau J, Maltais F, Eidelman DH, et al. CD8 positive T cells express IL-17 in patients with chronic obstructive pulmonary disease. Respir Res 2011;12:43.

276. Hong SC, Lee SH. Role of th17 cell and autoimmunity in chronic obstructive pulmonary disease. Immune Netw 2010;10(4):109-14.

277. Shapiro SD, Goldstein NM, Houghton AM, Kobayashi DK, Kelley D, Belaaouaj A. Neutrophil elastase contributes to cigarette smoke-induced emphysema in mice. Am J Pathol 2003;163(6):2329-35.

278. Voynow JA, Young LR, Wang Y, Horger T, Rose MC, Fischer BM. Neutrophil elastase increases MUC5AC mRNA and protein expression in respiratory epithelial cells. Am J Physiol 1999;276(5 Pt 1):L835-43.

279. Shen N, Wang J, Zhao M, Pei F, He B. Anti-interleukin-17 antibodies attenuate airway inflammation in tobacco-smoke-exposed mice. Inhal Toxicol 2011;23(4):212-8.

280. Doe C, Bafadhel M, Siddiqui S, Desai D, Mistry V, Rugman P, et al. Expression of the T helper 17-associated cytokines IL-17A and IL-17F in asthma and COPD. Chest 2010;138(5):1140-7.

281. Shan M, Cheng HF, Song LZ, Roberts L, Green L, Hacken-Bitar J, et al. Lung myeloid dendritic cells coordinately induce TH1 and TH17 responses in human emphysema. Sci Transl Med 2009;1(4):4ra10.

282. Zhang J, Chu S, Zhong X, Lao Q, He Z, Liang Y. Increased expression of CD4+IL-17+ cells in the lung tissue of patients with stable chronic obstructive pulmonary disease (COPD) and smokers. Int Immunopharmacol 2013;15(1):58-66.

283. O'Donnell R, Breen D, Wilson S, Djukanovic R. Inflammatory cells in the airways in COPD. Thorax 2006;61(5):448-54.

284. Freeman CM, Martinez FJ, Han MK, Washko GR, Jr., McCubbrey AL, Chensue SW, et al. Lung CD8+ T cells in COPD have increased expression of bacterial TLRs. Respir Res 2013;14:13.

285. Lee BJ, Moon HG, Shin TS, Jeon SG, Lee EY, Gho YS, et al. Protective effects of basic fibroblast growth factor in the development of emphysema induced by interferon-gamma. Exp Mol Med 2011;43(4):169-78.

286. Stampfli MR, Anderson GP. How cigarette smoke skews immune responses to promote infection, lung disease and cancer. Nat Rev Immunol 2009;9(5):377-84.

287. Feng Y, Kong Y, Barnes PF, Huang FF, Klucar P, Wang X, et al. Exposure to cigarette smoke inhibits the pulmonary T-cell response to influenza virus and Mycobacterium tuberculosis. Infect Immun 2011;79(1):229-37.

288. Lugade AA, Bogner PN, Thatcher TH, Sime PJ, Phipps RP, Thanavala Y. Cigarette smoke exposure exacerbates lung inflammation and compromises immunity to bacterial infection. J Immunol 2014;192(11):5226-35.

289. Petruccioli E, Petrone L, Vanini V, Sampaolesi A, Gualano G, Girardi E, et al. IFNgamma/TNFalpha specific-cells and effector memory phenotype associate with active tuberculosis. J Infect 2013;66(6):475-86.

290. Prezzemolo T, Guggino G, La Manna MP, Di Liberto D, Dieli F, Caccamo N. Functional Signatures of Human CD4 and CD8 T Cell Responses to Mycobacterium tuberculosis. Front Immunol 2014;5:180.

291. Caccamo N, Guggino G, Joosten SA, Gelsomino G, Di Carlo P, Titone L, et al. Multifunctional CD4(+) T cells correlate with active Mycobacterium tuberculosis infection. Eur J Immunol 2010;40(8):2211-20.

292. Cosmi L, Maggi L, Santarlasci V, Liotta F, Annunziato F. T helper cells plasticity in inflammation. Cytometry A 2014;85(1):36-42.

293. Chu S, Zhong X, Zhang J, Lao Q, He Z, Bai J. The expression of Foxp3 and ROR gamma t in lung tissues from normal smokers and chronic obstructive pulmonary disease patients. Int Immunopharmacol 2011;11(11):1780-8.

294. Mikko M, Forsslund H, Cui L, Grunewald J, Wheelock AM, Wahlstrom J, et al. Increased intraepithelial (CD103+) CD8+ T cells in the airways of smokers with and without chronic obstructive pulmonary disease. Immunobiology 2013;218(2):225-31.

295. Isajevs S, Taivans I, Strazda G, Kopeika U, Bukovskis M, Gordjusina V, et al. Decreased FOXP3 expression in small airways of smokers with COPD. Eur Respir J 2009;33(1):61-7.

296. Trinchieri G. Interleukin-10 production by effector T cells: Th1 cells show self control. J Exp Med 2007;204(2):239-43.

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

298. Roos-Engstrand E, Pourazar J, Behndig AF, Bucht A, Blomberg A. Expansion of CD4+CD25+ helper T cells without regulatory function in smoking and COPD. Respir Res 2011;12:74.

299. Raffin C, Pignon P, Celse C, Debien E, Valmori D, Ayyoub M. Human memory Helios-FOXP3+ regulatory T cells (Tregs) encompass induced Tregs that express Aiolos and respond to IL-1beta by downregulating their suppressor functions. J Immunol 2013;191(9):4619-27.

300. Wang H, Peng W, Weng Y, Ying H, Li H, Xia D, et al. Imbalance of Th17/Treg cells in mice with chronic cigarette smoke exposure. Int Immunopharmacol 2012;14(4):504-12.

301. Harel-Meir M, Sherer Y, Shoenfeld Y. Tobacco smoking and autoimmune rheumatic diseases. Nat Clin Pract Rheumatol 2007;3(12):707-15.

302. Jawaheer D, Gregersen PK. Rheumatoid arthritis. The genetic components. Rheum Dis Clin North Am 2002;28(1):1-15, v.

303. Odoardi F, Sie C, Streyl K, Ulaganathan VK, Schlager C, Lodygin D, et al. T cells become licensed in the lung to enter the central nervous system. Nature 2012;488(7413):675-9.

304. Altintas A, Demir T, Ikitimur HD, Yildirim N. Pulmonary function in multiple sclerosis without any respiratory complaints. Clin Neurol Neurosurg 2007;109(3):242-6.

305. Lovett-Racke AE, Yang Y, Racke MK. Th1 versus Th17: are T cell cytokines relevant in multiple sclerosis? Biochim Biophys Acta 2011;1812(2):246-51.

306. Spath S, Becher B. T-bet or not T-bet: taking the last bow on the autoimmunity stage. Eur J Immunol 2013;43(11):2810-3.

307. Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, Esiri MM, et al. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. Am J Pathol 2008;172(1):146-55.

308. Stromnes IM, Cerretti LM, Liggitt D, Harris RA, Goverman JM. Differential regulation of central nervous system autoimmunity by T(H)1 and T(H)17 cells. Nat Med 2008;14(3):337-42.

309. Eisenstein EM, Williams CB. The T(reg)/Th17 cell balance: a new paradigm for autoimmunity. Pediatr Res 2009;65(5 Pt 2):26R-31R.

310. Sweeney CM, Lonergan R, Basdeo SA, Kinsella K, Dungan LS, Higgins SC, et al. IL-27 mediates the response to IFN-beta therapy in multiple sclerosis patients by inhibiting Th17 cells. Brain Behav Immun 2011;25(6):1170-81.

311. Chen M, Chen G, Deng S, Liu X, Hutton GJ, Hong J. IFN-beta induces the proliferation of CD4+CD25+Foxp3+ regulatory T cells through upregulation of GITRL on dendritic cells in the treatment of multiple sclerosis. J Neuroimmunol 2012;242(1-2):39-46.

312. de Andres C, Aristimuno C, de Las Heras V, Martinez-Gines ML, Bartolome M, Arroyo R, et al. Interferon beta-1a therapy enhances CD4+ regulatory T-cell function: an ex vivo and in vitro longitudinal study in relapsing-remitting multiple sclerosis. J Neuroimmunol 2007;182(1-2):204-11.

313. Ramos-Cejudo J, Oreja-Guevara C, Stark Aroeira L, Rodriguez de Antonio L, Chamorro B, Diez-Tejedor E. Treatment with natalizumab in relapsing-remitting multiple sclerosis patients induces changes in inflammatory mechanism. J Clin Immunol 2011;31(4):623-31.

314. Komatsu N, Okamoto K, Sawa S, Nakashima T, Oh-hora M, Kodama T, et al. Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis. Nat Med 2014;20(1):62-8.

315. Dominguez-Villar M, Baecher-Allan CM, Hafler DA. Identification of T helper type 1-like, Foxp3+ regulatory T cells in human autoimmune disease. Nat Med 2011;17(6):673-5.

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

317. Uhlig HH, Coombes J, Mottet C, Izcue A, Thompson C, Fanger A, et al. Characterization of Foxp3+CD4+CD25+ and IL-10-secreting CD4+CD25+ T cells during cure of colitis. J Immunol 2006;177(9):5852-60.

318. Ranatunga DC, Ramakrishnan A, Uprety P, Wang F, Zhang H, Margolick JB, et al. A protective role for human IL-10-expressing CD4+ T cells in colitis. J Immunol 2012;189(3):1243-52.

319. Joetham A, Takeda K, Taube C, Miyahara N, Matsubara S, Koya T, et al. Naturally occurring lung CD4(+)CD25(+) T cell regulation of airway allergic responses depends on IL-10 induction of TGF-beta. J Immunol 2007;178(3):1433-42.

320. Hawrylowicz CM, O'Garra A. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. Nat Rev Immunol 2005;5(4):271-83.

321. Sawant DV, Vignali DA. Once a Treg, always a Treg? Immunol Rev 2014;259(1):173-91.

322. Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor? Immunity 2009;30(5):626-35.

323. Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. Autoimmun Rev 2014;13(6):668-77.

324. Frisullo G, Nociti V, Iorio R, Patanella AK, Caggiula M, Marti A, et al. Regulatory T cells fail to suppress CD4T+-bet+ T cells in relapsing multiple sclerosis patients. Immunology 2009;127(3):418-28.