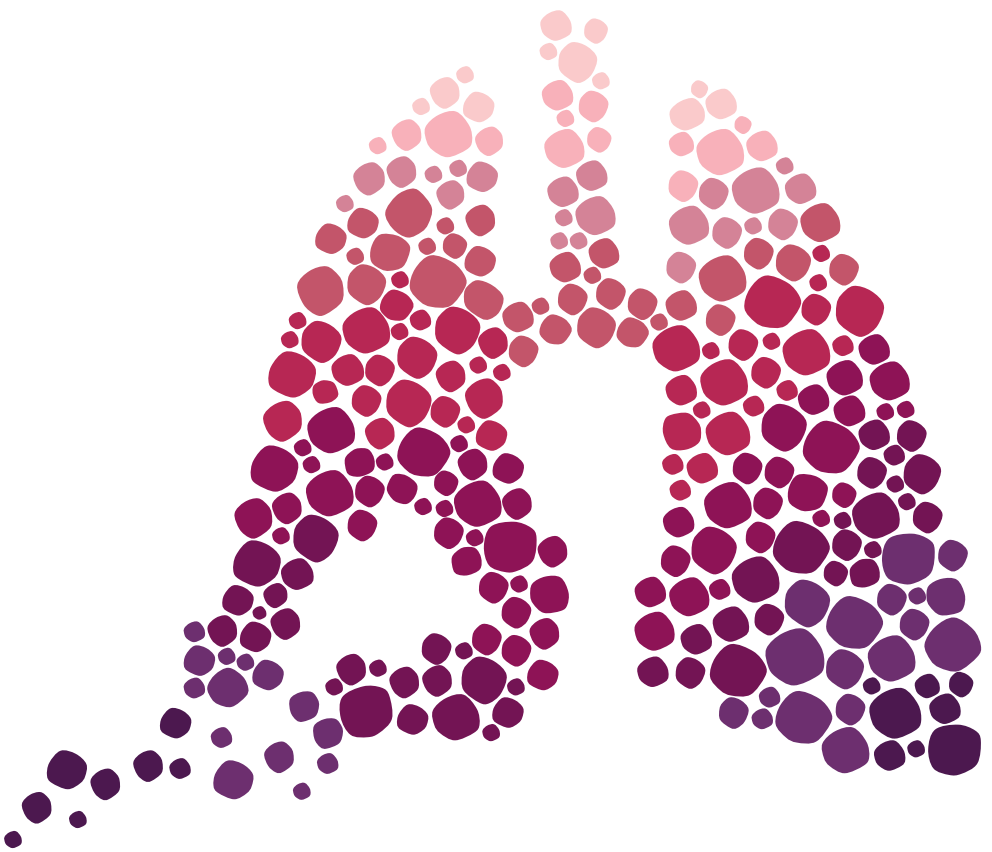
Thesis for doctoral degree (Ph.D.)
2018

THE BLIND SPOT

Inflammatory Pathways and Specific Antigens in Clinical Phenotypes of Pulmonary Sarcoidosis



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INFLAMMATORY PATHWAYS AND SPECIFIC ANTIGENS IN CLINICAL PHENOTYPES OF PULMONARY SARCOIDOSIS

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THE BLIND SPOT

Inflammatory Pathways and Specific Antigens in Clinical Phenotypes of Pulmonary Sarcoidosis

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Only dull people are brilliant at breakfast.

- Oscar Wilde

ABSTRACT

Sarcoidosis is a granulomatous multisystem disorder of unknown aetiology, primarily manifesting in the lungs. The disease appears in two forms, which are mainly distinguished based on clinical criteria; Löfgren's syndrome (LS) presents with acute onset, usually fever, active inflammation and characteristic symptoms such as bilateral hilar lymphadenopathy (BHL), erythema nodosum and/or ankle arthritis, but is also associated with spontaneous resolution and a good prognosis. In contrast, "non-LS" comprises a more heterogeneous patient group, with common characteristics being an insidious onset, slower disease progression and a higher risk of developing chronic disease, extrapulmonary manifestations and eventually pulmonary fibrosis. Despite advances in genetics, epidemiology, immunology and therapeutics during the past decades, the disease-triggering antigen(s) and the immunological mechanisms underlying different clinical phenotypes remain a "blind spot" – something that is known to exist but that the eye is, as yet, unable to perceive. Previous studies have shown the human leukocyte antigen (HLA)-DR allele *HLA-DRB1*03* to associate closely with LS and a favourable clinical outcome. In these patients, local expansion of $CD4^+$ T cells carrying the specific T cell receptor (TCR) variable (V) segment Vα2.3 in the lungs suggests specific antigen recognition, making these cells ideal tools in the quest for disease-triggering factors in sarcoidosis.

The aims of this thesis were therefore to investigate on one hand the phenotype, functionality and ultimately antigen-specificity of TCR-specific $CD4^+$ T cells, and on the other to improve understanding of immunological mechanisms underlying distinct clinical phenotypes of sarcoidosis.

In HLA-DRB1*03^+ patients with sarcoidosis, Vα2.3 was found to preferentially pair with the Vβ22 TCR segment on $CD4^+$ T cells in the lung. Expression of activation markers on these cells indicated an advanced state of differentiation, consistent with prolonged antigen exposure, and a high degree of clonality, marked by appearance of identical and near-identical TCR α and β CDR3 sequences between patients. In addition, molecular modelling of the TCR Vα2.3/Vβ22-HLA-DRB1*03 complex revealed an ideal fit into the peptide-binding cleft of a peptide derived from the carboxyl (C)-terminal of cytoskeletal protein vimentin, implicating vimentin as a potential (auto)antigen in sarcoidosis and suggesting especially LS to harbour traits of autoimmunity. Accordingly, anti-vimentin IgG and IgA antibodies were detected in the lungs of sarcoidosis patients to a higher degree

than in healthy individuals, and correlated with expression of the Vα2.3/Vβ22 TCR. HLA-DRB1*03^{+} patients also demonstrated lower total Ig counts, but stronger reactivity towards the vimentin C-terminal, ultimately suggesting a more antigen-driven, but less aggressive, cooperative T-B cell response in HLA-DRB1*03-mediated disease.

Particularly in LS patients, CD4^{+} T cells in the lung demonstrated simultaneous expression of T helper (T_H) 1 transcriptional regulator T-bet and T_H17 counterpart RORγT, which intriguingly correlated with non-chronic disease, and responded to stimuli with a broad array of cytokines, including interleukin (IL)-10, IL-2, IL-17A and IL-22, in addition to interferon (IFN)-γ, the main cytokine produced in non-LS patients. Moreover, expression of inhibitory receptors CTLA-4 and PD-1 indicated a higher degree of regulatory capacity compared to non-LS CD4^{+} T cells, where elevated expression of HLA-DR, CD127 and CD39 pointed towards a more pronounced effector profile. The discovery of marked CD8^{+} T cell proliferation in both patient groups provides further evidence for a hitherto unappreciated role of CD4^{+} T cells in restriction of cytotoxic T cell activity to prevent tissue damage in sarcoidosis, with loss of CTLA-4- and PD-1-mediated inhibition in non-LS patients exacerbating the risk of developing chronic disease and permanent scarring. Combined with reduced expression of adhesion marker CD44 in LS, this detailed characterisation outlines a possible mechanism for spontaneous resolution of granulomas.

The collected findings of this thesis suggest fundamental differences in CD4^{+} T cell biology that may be of key importance for disease resolution and progression, respectively, in clinical phenotypes of pulmonary sarcoidosis. Moreover, the results presented constitute an important step forward in the search for disease-specific antigens, and provide incentive for further exploration of sarcoidosis, and LS in particular, as an autoimmune condition, at least partly driven by cognate T and B cell reactivity to vimentin. Hopefully, these discoveries may shed further light upon the still-elusive enigma of sarcoidosis, act as a foundation for continued investigation of disease-instigating factors, and ultimately contribute to reconsideration of current disease classification.

POPULAR SCIENCE SUMMARY

The Blind Spot concerns sarcoidosis, which is an inflammatory lung disease of unknown origin. It can also affect several other organs, such as the spleen, liver, kidneys, skin, eyes, or, most severely, the heart or central nervous system (CNS). Disease occurrence varies with geographical region, but is particularly high in Northern Europe; the annual incidence in Sweden is approximately 1200, with around 16,000 individuals currently being estimated to suffer from sarcoidosis. However, symptomatic presentation is highly variable, and many cases likely remain unresolved. Sarcoidosis presents in two main forms, both of which are characterised by the presence of so-called granulomas, large collections of immune cells, in the affected organs. In addition, an expansion of T cells, a branch of the adaptive immune system, is observed in the lungs and used as a diagnostic determinant. Löfgren's syndrome (LS) is an acute condition characterised by fever, enlarged lymph nodes and marked inflammatory reactions on the lower extremities. "Non-LS" comprises a much more diverse group, with patients usually experiencing insidious onset, slower progression, and a higher frequency of symptomatic manifestation from other organs than the lungs. Importantly, patients with LS usually have a good prognosis, with the disease resolving spontaneously within a few months up to two years. In contrast, non-LS patients have a higher risk of developing chronic disease with permanent tissue damage and eventually pulmonary fibrosis, which, together with cardiac and CNS involvement, are the most common causes of sarcoidosis-associated mortality. As yet, therapeutic options are limited and remain unspecific, and most patients receive either no treatment, or merely symptomatic relief, for their disease.

Previous studies have shown the two conditions to differ markedly in genetic background, with genes governing uptake and recognition of immune-stimulatory substances, so-called antigens, deviating substantially between LS and non-LS. Specifically, presence of genetic variant *HLA-DRB1*03* is closely linked to LS and a good prognosis. HLA molecules are responsible for presentation of protein-derived segments, or peptides, to T cells for recognition and subsequent activation. However, the exact immunological pathways underlying the two disease conditions remain unknown, a "blind spot", as do the identity of the disease-triggering substance(s). The aim was therefore to conduct a search for disease-specific antigens, as well as to perform a detailed investigation of T cell responses in the lungs of sarcoidosis patients, ultimately striving to identify factors that affect disease resolution or progression in LS and non-LS patients, respectively.

The results presented in this thesis show an increase of T cells expressing a particular T cell receptor (TCR) for antigen recognition in the lungs of sarcoidosis patients positive for *HLA-DRB1*03*. These cells express markers associated with T cell maturation and exposure to antigenic stimulation, suggesting that these cells could be used as tools for antigen discovery. Using the previously known structure of the HLA-DRB1*03 molecule and the newly identified TCR, a molecular model was generated to identify structural characteristics of the presented antigenic peptide. This approach indicated that vimentin, a protein participating in maintenance of the cellular cytoskeleton, could be a candidate antigen in sarcoidosis, thereby suggesting the disease to have traits of autoimmunity. Further studies revealed reactivity not only of T cells, but also of B cells, which are responsible for antibody production, towards vimentin. This reactivity was specifically localised to the lung and most prominent in HLA-DRB1*03^{+} patients.

Overall, these studies demonstrate several notable differences in T cell biology between LS and non-LS patients. Most prominently, T cells from LS patients exhibited a more diverse functional repertoire, maintaining both activating and inhibitory functions. An efficient, but self-restrictive immune response could be related to both the acute disease onset and spontaneous resolution observed in this patient group. In contrast, non-LS patients showed signs of a more destructive immune reactivity, possibly contributing to propagated inflammation that may result in chronic disease, tissue destruction and eventually fibrotic scarring. It is possible that, in time, these two conditions will come to be considered as separate disease entities.

In conclusion, this thesis provides further insights into complex immunological mechanisms in the lungs of sarcoidosis patients, and identifies factors related to antigen recognition and immune regulation as particularly important in the development of LS or non-LS disease, respectively. Enhanced understanding of subtle molecular differences between the two conditions may contribute to improved prognostic assessment in a clinical setting, as well as advances in future, more targeted therapeutic strategies. Most prominently, the identification of TCR-specific T cells in HLA-DRB1*03^{+} LS patients provides a theoretical and methodological foundation for further investigation of disease origin, both in LS and non-LS, with the aim of resolving the “blind spot” of sarcoidosis.

LIST OF SCIENTIFIC PAPERS

I. Grunewald J, **Kaiser Y**, Ostadkarampour M, Rivera NV, Vezzi F, Lötstedt B, Olsen RA, Sylwan L, Lundin S, Käller M, Sandalova T, Ahlgren KM, Wahlström J, Achour A, Ronninger M, Eklund A.
T-cell receptor-HLA-DRB1 associations suggest specific antigens in pulmonary sarcoidosis.
Eur Respir J. 2016 Mar;47(3):898-909.
Editorial comment in *Eur Respir J.* 2016 Mar;47(3):707-9.

II. **Kaiser Y**, Lepzien R, Kullberg S, Eklund A, Smed-Sörensen A, Grunewald J.
Expanded lung T-bet^{+}RORγT^{+} CD4^{+} T-cells in sarcoidosis patients with a favourable disease phenotype.
Eur Respir J. 2016 Aug;48(2):484-94.

III. **Kaiser Y**, Lakshmikanth T, Chen Y, Mikes J, Eklund A, Brodin P, Achour A, Grunewald J.
Mass cytometry identifies distinct lung CD4^{+} T cell patterns in Löfgren's syndrome and non-Löfgren's syndrome sarcoidosis.
Front Immunol. 2017 Sep 12;8:1130.

IV. Mitchell AM, **Kaiser Y**, Falta MT, Munson DJ, Landry LG, Eklund A, Nakayama M, Slansky JE, Grunewald J, Fontenot AP.
Shared αβ TCR usage in lungs of sarcoidosis patients with Löfgren's syndrome.
J Immunol. 2017 Oct 1;199(7):2279-2290.

V. Kinloch AJ*, **Kaiser Y***, Wolfgeher D, Eklund A, Clark MR$^{\#}$, Grunewald J$^{\#}$.
***In situ* humoral immunity to vimentin in HLA-DRB1*03^{+} patients with pulmonary sarcoidosis.**
Submitted.

* These authors contributed equally

$^{\#}$ These authors contributed equally

ASSOCIATED PAPERS NOT INCLUDED IN THE THESIS

I. Karakaya B*, **Kaiser Y***, van Moorsel CHM#, Grunewald J#.
Löfgren's syndrome: diagnosis, management, and disease pathogenesis.
Semin Respir Crit Care Med. 2017 Aug;38(4):463-476. *Review*.

II. Miedema JR*, **Kaiser Y***, Broos CE, Wijsenbeek MS, Grunewald J, Kool M.
Th17-lineage cells in pulmonary sarcoidosis and Löfgren's syndrome: Friend or foe?
J Autoimmun. 2018 Feb;87:82-96. *Review*.

* These authors contributed equally

# These authors contributed equally

CONTENTS

LIST OF ABBREVIATIONS

ACE	Angiotensin-converting enzyme
AID	Activation-induced deaminase
ANOVA	Analysis of variance
APC	Antigen-presenting cell
AVA	Anti-vimentin antibody
BAFF	B cell-activating factor
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
BCR	B cell receptor
Bcl-6	B cell lymphoma protein 6
BHL	Bilateral hilar lymphadenopathy
BTNL2	Butyrophilin-like 2
CBD	Chronic beryllium disease
CD	Cluster of differentiation
CDR	Complementarity determining region
CCR	C-C chemokine receptor
CLIP	Class II-associated invariant chain peptide
CNS	Central nervous system
CTL	Cytotoxic T lymphocyte
CTLA-4	Cytotoxic T lymphocyte-associated antigen-4
CXCL	C-X-C motif ligand
CXCR	C-X-C chemokine receptor
DAMP	Danger-associated molecular pattern
DC	Dendritic cell
DLCO	Diffusing capacity of carbon monoxide
EBV	Epstein-Barr virus
ELISA	Enzyme-linked immunosorbent assay
ELISpot	Enzyme-linked immunospot
ePCR	Emulsion PCR
ER	Endoplasmic reticulum
ESAT-6	6 kDa early secretory antigenic target
FACS	Fluorescence-activated cell sorting
FDC	Follicular dendritic cell
FDR	False discovery rate
FEV_1	Forced expiratory volume in 1 s
FoxP3	Forkhead box protein 3

FSC	Forward scatter
FVC	Forced vital capacity
GC	Germinal centre
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HAART	Highly active anti-retroviral therapy
HLA	Human leukocyte antigen
ICAM-1	Intercellular adhesion molecule-1
ICOS	Inducible co-stimulator
ICP-MS	Inductively coupled plasma-mass spectroscopy
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
ILD	Interstitial lung disease
IPF	Idiopathic pulmonary fibrosis
LFA-1	Leukocyte function-associated antigen-1
LS	Löfgren's syndrome
MGC	Multinucleated giant cell
MHC2TA	Major histocompatibility complex 2 transactivator
MIF	Macrophage inhibitory factor
mKatG	Mycobacterial catalase-peroxidase
MS	Multiple sclerosis
mTOR	Mechanistic target of rapamycin
NGS	Next-Generation Sequencing
NK	Natural killer
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PD-1	Programmed cell death protein-1
PMT	Photomultiplier tube
PPD	Purified protein derivative
PRR	Pattern-recognition receptor
PVDF	Polyvinylidene difluoride
RA	Rheumatoid arthritis
RORγT	Retinoic acid-related orphan receptor γ thymocyte-specific
SAA	Serum amyloid A
SLE	Systemic lupus erythematosus
SSC	Side scatter

STAT	Signal transducer and activator of transcription
TAP	Transporter associated with antigen processing
T-bet	T-box transcription factor
TCR	T cell receptor
T_{FH}	Follicular T helper
TGF	Transforming growth factor
T_H	T helper
TIM	T cell immunoglobulin mucin domain
TLC	Total lung capacity
TNF	Tumour necrosis factor
TOF	Time-of-flight
T_{REG}	Regulatory T cell
t-SNE	*t*-distributed stochastic neighbourhood embedding
VC	Vital capacity

1 INTRODUCTION

1.1 THE IMMUNE SYSTEM

The human immune system is a complex network of defence mechanisms that act in conjunction to protect the host from disease. It involves specialised immune cells that target harmful, foreign substances for destruction, as well as peripheral organs and tissue barriers that prevent the entry of invading microbes. Divided into two major branches, the innate and the adaptive, the immune system ensures both rapid responsiveness, as well as gradual development of a highly efficient and targeted elimination of pathogens (Fig. 1).

Innate, or natural, immunity constitutes the inherent protective mechanism that guards the body against microbial invasion. The first line of defence is comprised of physical barriers at mucosal surfaces such as the skin, respiratory and gastrointestinal tracts, where cells and natural antibiotics in the epithelium block the entry of foreign substances. If the epithelial layer is breached, the presence of microbes in the tissue or circulation will alert phagocytic cells, most prominently tissue-resident macrophages, neutrophils and circulating monocytes. Innate immune cells react rapidly (within hours) to common microbial structures, termed pathogen-associated molecular patterns (PAMPs), or damage-associated components released by necrotic cells (DAMPs), ensuring responsiveness to a broad range of possible detrimental agents. Engagement of membrane-bound pattern-recognition receptors (PRRs) results in downstream activation of transcription factors promoting upregulation of endothelial adhesion molecules and release of extracellular signalling mediators, including cytokines such as interleukin (IL)-1β, IL-6, IL-12, IL-18, tumour necrosis factor (TNF)-α and chemokines for recruitment of cells to the site of inflammation. Upon activation, natural killer (NK) cells, a distinct set of bone marrow-derived lymphocytes, mediate lysis of infected cells and secretion of interferon (IFN)-γ, a cytokine essential for driving macrophage activation, engulfment and destruction of microorganisms. Finally, dendritic cells (DCs) and other antigen-presenting cells (APCs), are essential in orchestrating the induction of adaptive immune responses through the efficient uptake, processing and presentation of foreign antigens, as well as secretion of cytokines that promote differentiation of effector cells (1).

Adaptive, or acquired, immunity is mediated by lymphocytes, most prominently T and B cells. In contrast to innate immune cells, lymphocytic responses emerge

later (within days) following the initial triggering event, but somatic recombination of germline-encoded receptor gene segments allows for superior diversity and enhanced specificity of responses to antigen. The low number of cells with a particular antigen specificity, in combination with the high variability in receptor composition, generates an extremely diverse cellular repertoire that is able to respond to a vast number and variety of antigens. As opposed to innate immune cells, this reactivity may also include non-infectious agents as well as microbes, and, in some instances, even self-antigens. Following recognition of an antigen presented on an APC, mature activated lymphocytes undergo clonal expansion to generate multiple progeny cells with the same antigen specificity. Elimination of the antigenic source by effector cells, and thereby removal of stimuli essential for effector cell survival, eventually results in apoptosis of the majority of clonally expanded cells, allowing the immune system to resume homeostasis after antigen clearance. The residual fraction of effector cells remain in the form of long-lived memory cells that are able to mount rapid and specific recall responses to previously encountered pathogens, enabling targeted and efficient clearance of antigen upon re-infection (1, 2).

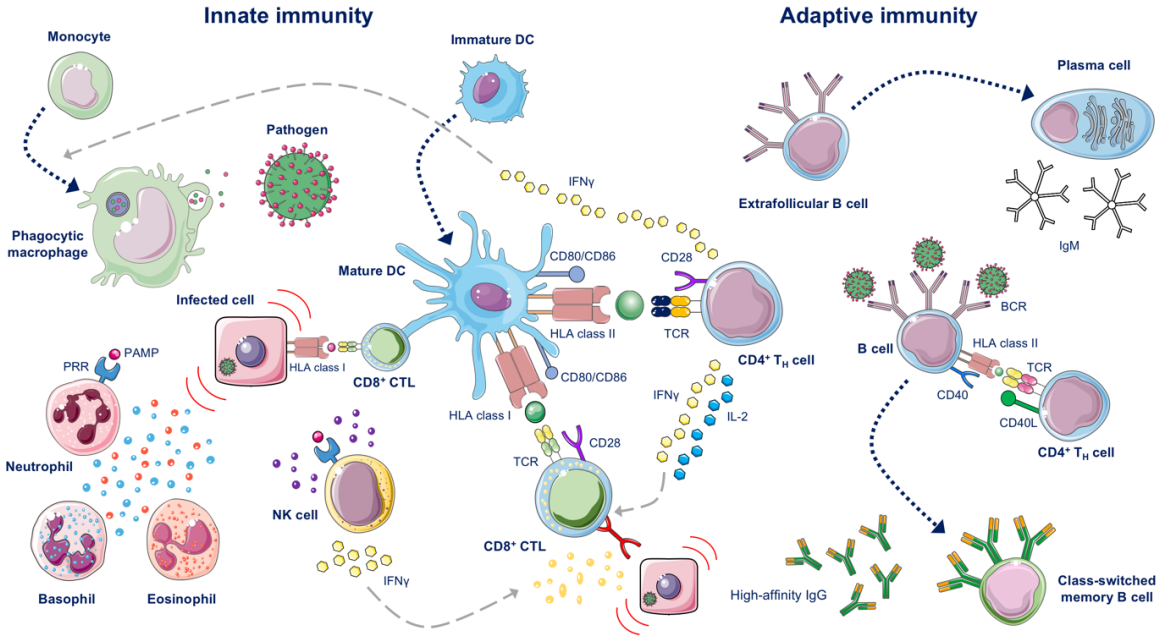

Figure 1 | Major components of the human immune system. The innate and adaptive branches of the immune system together orchestrate a coordinated cascade that ensures both rapid responsiveness to common pathogenic patterns, as well as highly efficient, antigen-specific cellular and humoral responses that ultimately result in elimination of the antigenic source and generation of long-lived memory cells. The figure offers a schematic overview of cell types, receptors and secreted signalling mediators central to innate and adaptive immunity.

1.2 T LYMPHOCYTES

T cells, which constitute the cellular branch of the adaptive immune system, develop in the bone marrow and migrate to the thymus for maturation. All T cells are equipped with a T cell receptor (TCR) designed and selected for recognition of peptide antigens derived from foreign, potentially harmful protein structures, e.g. bacteria, viruses and parasites. Naïve T cells continuously circulate through secondary lymphoid organs in search for antigen, which, when recognised, will result in their activation and differentiation into effector cells. These cells then migrate to peripheral sites to perform effector functions, e.g. initiation of antibody production by B cells and priming of macrophage activity. Cells carrying the cluster of differentiation (CD) 4 co-receptor on their surface are termed T helper (T_H) cells, and serve to orchestrate the immune response through activation of B cells, phagocytic cells, and cytotoxic $CD8^+$ T cells. These functions are mediated both through direct cell-cell interactions and release of cytokines and chemokines. $CD4^+$ T cells recognise protein antigens presented on human leukocyte antigen (HLA) class II molecules on the surface of APCs such as DCs, macrophages and B cells (3). $CD8^+$ T cells, also termed cytotoxic T lymphocytes (CTLs), instead respond to HLA class I-presented peptides and frequently require concomitant activation of $CD4^+$ T cells and their subsequent secretion of stimulatory cytokines. Activation of CTLs results in granule exocytosis, and the release of perforin and granzymes into the extracellular space. Perforin inserts into the cell membrane to form pores that facilitate entry of granzymes, which in turn engage the apoptotic cascade, directly killing the infected, antigen-expressing target cell.

1.3 HLA IN IMMUNITY AND DISEASE

HLA class I molecules are expressed on all nucleated cells and accommodate peptides averaging 8-11 amino acids in length, which they present to $CD8^+$ T cells. These antigens are often derived from cytosolic proteins synthesised within the cells, e.g. viral proteins. Class II molecules are expressed on the surface of professional APCs and comprise five main binding pockets at positions P1, P4, P6, P7 and P9, which bind peptides of 13-15 amino acid residues with broad specificity, i.e. not all peptide-binding pockets have to be occupied in order for a peptide to be recognised (4). These peptides are usually internalised from the extracellular environment and are presented to $CD4^+$ T cells. In addition, the process of cross-presentation, which occurs e.g. when an APC has ingested an infected cell, allows peptides derived from the same intracellular protein to be presented both on class I molecules to $CD8^+$ T cells and on class II molecules for

recognition by $CD4^+$ T cells. HLA molecules present only one peptide at a time, but each molecule is capable of presenting multiple different peptides, as long as the binding pockets can accommodate the anchor residues of the peptide. Other residues project upward for interaction with and recognition by the TCR (1).

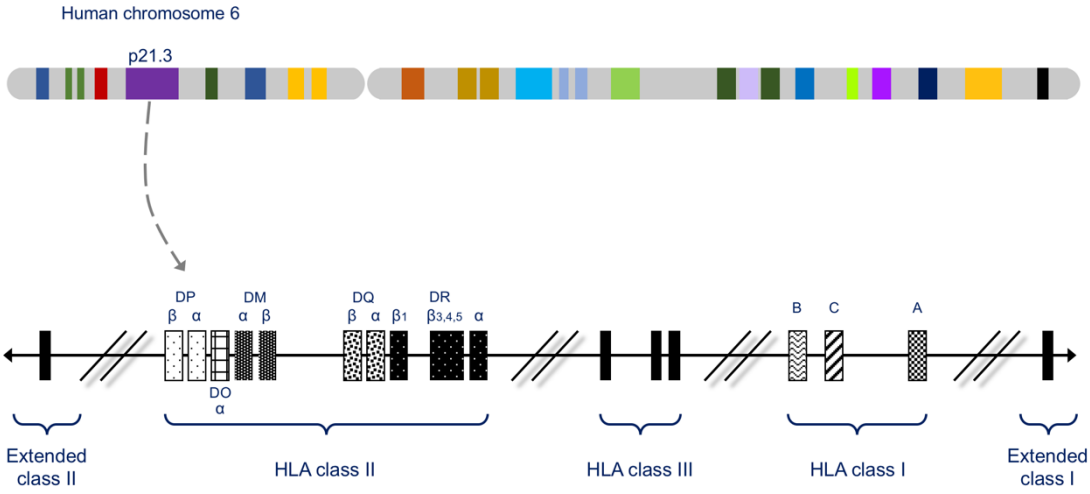

Figure 2 | The HLA genetic region. In humans, the HLA loci are located in the p21.3 section on chromosome 6. In addition to the major and minor HLA class I and class II alleles, this region comprises HLA class III alleles, which encode, among others, gene products involved in transcriptional regulation, immune activity, protein-protein interactions, transport and signalling, as well as serum protein mediators and TNF family cytokines.

In humans, the HLA loci are located on chromosome 6 and comprise HLA-A, -B, and –C (class I) as well as HLA-DP, -DQ and -DR (class II) alleles. In addition, minor class I alleles HLA-E, -F and -G and HLA class II alleles HLA-DM and -DO, associated with internal antigen processing, are also encompassed in the same genetic region (Fig. 2). Each individual inherits one maternal and one paternal copy of the same HLA allele. Hence, for HLA class I, which exists in three major forms, any given cell can express six different class I molecules. For class II, every individual inherits one pair of HLA-DP genes (DPA1 and DPB1, for the α and β chains, respectively), one pair of HLA-DQ (DQA1 and DQB1), one HLA-DRα (DRA1) and one or two HLA-DRβ genes (DRB1 and DRB3, -4 or -5) from each parent. The DR β chain is encoded by four different loci, but no more than three functional loci are present in any given individual. HLA-DRB1 genes encode a large number of functionally variable products, while HLA-DRB3, HLA-DRB4 and HLA-DRB5 show decreasing variability and are tightly associated with presence of certain DRB1 alleles (5, 6). Distinct DR and DQ alleles are in so-called linkage disequilibrium, meaning that they are frequently inherited together, and the HLA class I and II alleles present on each chromosome together constitute the genetic

haplotype. The numerous possibilities for combination inherent to each individual and the highly polymorphic spread of HLA alleles ensure that some members of an outbred population will always be able to present, recognise and mount an immune response to any particular pathogen (1).

1.4 ANTIGEN PRESENTATION

The distinction between HLA class I and class II molecules and the range of cells expressing a respective class of molecules ensure that the immune response mounted is ideally suited to manage the current threat. For extracellular antigens, this specifically involves activation of $CD4^+$ T cells that in turn promote antibody production by B cells and activation of phagocytosis by macrophages. In contrast, CTL-mediated direct lysis of peptide-presenting infected cells is a more efficient strategy in the case of antigens present in the cytosol.

Antigen processing follows two main pathways depending on the route of uptake. Degradation of cytosolic proteins by the proteasome is followed by loading onto the membrane-bound protein transporter associated with antigen processing (TAP) in the endoplasmic reticulum (ER), where HLA molecules are being synthesised. TAP facilitates transport of peptides into the ER, and by linkage to the protein tapasin, which attaches to newly synthesised HLA class I molecules, the empty HLA molecules are poised to receive peptides entering the ER. Loading of a peptide stabilises the complex and triggers release from TAP, whereupon the peptide-class I complex can be transported to the cell surface through exocytosis. For presentation of extracellular peptides ingested by APCs, proteins are degraded under the acidic conditions of the endosomal and lysosomal compartments. HLA class II molecules produced in the ER carry an invariant chain, where the section termed the class II-associated invariant chain peptide (CLIP) attaches tightly to the peptide-binding cleft. The occupation of the peptide groove by CLIP ensures that intracellular peptides transported into the ER by TAP can bind their intended class I molecules, and are not accidentally paired with class II. Instead, CLIP-bound class II molecules are dispatched in exocytic vesicles that eventually fuse with the late endosomal-lysosomal vesicles containing proteolytically degraded extracellular peptides. There, the DM protein mediates exchange of CLIP for surrounding higher-affinity peptides, binding of which stabilises the class II complex and enables its transport to the cell surface. Failure of peptide recognition results in degradation of empty molecules by vesicular proteases.

Importantly, a single protein may give rise to many different peptides, only a few of which are bound by the HLA; these peptides are referred to as immunodominant, as solely the HLA-bound peptides are able to initiate immune responses by T cells. The HLA combination in any given individual thus determines which peptides can be presented, and consequently the ability of that person to mount an immune response to a certain antigen. Once bound, peptides remain in the binding-cleft up to several days, increasing the possibility that a T cell specific for the presented peptide will recognise it. Engagement of the TCR in response to an HLA molecule and its peptide sustains surface expression of the complex, allowing downstream signalling through the TCR to occur (1, 7).

1.5 TCR SIGNALLING

Antigen recognition is mediated through several steps, the first of which being the recognition by a TCR of a peptide epitope for which it is specific. Most commonly, TCRs consist of an α and a β chain, each of which contain a constant and a variable domain. The α chain variable region is encoded by variable (V) and joining (J) gene segments; for the β chain, these are interspersed with additional diversity (D) segments. Somatic rearrangement of the germline repertoire and additional random nucleotide insertions and deletions between the V, D and J segments then facilitate the immense variability in TCR specificity that is required for recognition of a broad range of pathogens. The antigen-binding site itself comprises three complementarity determining regions (CDRs), of which CDR3 is the most variable and also the major contact point for antigen recognition. Signal transduction through the TCR into the cell also requires T cell-specific co-receptor CD3 and the ζ chain, which jointly generate an activation signal upon TCR recognition of HLA-peptide complexes (Fig. 3). Most TCRs bind the peptide-HLA complex with low affinity, and upon TCR binding, chemoattractant molecules are released to promote association of T cell integrin leukocyte function-associated antigen-1 (LFA-1) with its APC-bound ligand intercellular adhesion molecule-1 (ICAM-1). This mediates stable cellular adhesion and proximity for a prolonged period of time, allowing receptor and co-receptor engagement to yield a productive response. The T cell CD4 and CD8 co-receptors ensure accurate binding of the TCR to either HLA class II or class I molecules, respectively. The second signal of activation is then mediated by co-stimulatory molecules on the surface of the T cell and APC, namely interaction of activating co-receptor CD28 and CD40L on the T cell with CD80/CD86 and CD40, respectively, on the APC. This chain of events induces phosphorylation, docking and activation of downstream intracellular

kinases that eventually result in upregulation of adequate transcription factors in the nucleus, driving the effector response and subsequent T cell proliferation, differentiation, cytokine production, clonal expansion, and finally, cell migration to the site of inflammation (1).

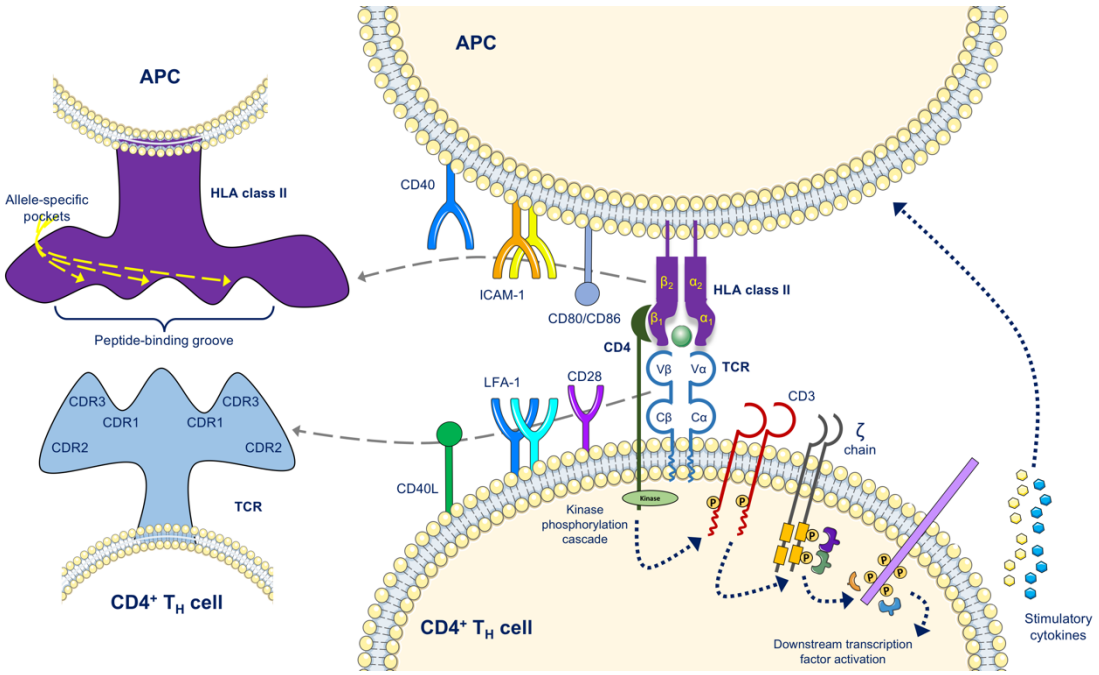

Figure 3 | Antigen presentation and TCR signalling. Detail of the interface between an APC and a CD4⁺ T cell and the main components and co-receptors involved in antigen presentation and subsequent downstream TCR signalling. Successful recognition of an HLA class II-presented peptide by the TCR and simultaneous recognition of HLA class II by the CD4 co-receptor trigger an intracellular phosphorylation cascade mediated by kinases and adaptor proteins associated with T cell co-receptors CD3 and ζ, ultimately resulting in activation of transcription factors involved in inflammatory processes. Concomitant association of activating co-receptor CD28 on the T cell with its ligands CD80/CD86 on the APC, T cell CD40L and APC CD40, as well as T cell secretion of activating cytokines, provide a stimulatory environment for continued interaction and signalling. In addition, attachment of integrins LFA-1 and ICAM-1 to one another ensure the immunological synapse is maintained. The α and β CDR3 regions of the TCR comprise the most amino acid variability and carry the main responsibility for antigen recognition.

1.6 CD4⁺ T CELL SUBSETS

Upon activation, CD4⁺ T cells can differentiate into various subsets with different characteristics and functions, the most commonly discussed termed T_H1, T_H2 and T_H17, as well as regulatory T cells (T_{REG}s). T_H1 cells are characterised by expression of transcription factors T-bet, signal transducers and activators of transcription (STAT)1 and STAT4, production of cytokines IFNγ, IL-2 and TNF-α, activation of macrophages, as well as induction of antibody class-switching in B cells. T_H1 differentiation is induced by IL-12, IL-18 and IFNγ, and constitutes an important protective response against intracellular microbes (3, 8, 9).

T_H2 cells are polarised through IL-4 signalling via STAT6, express transcription factor GATA3 and produce IL-4, IL-5 and IL-13 (10), which induce eosinophil activation and IgE, IgG_1, IgG_4 and IgA production by B cells. T_H2 cells are major mediators of asthma and allergic responses, but also serve a physiological role in protection against helminths (3, 11).
T_H17 cells constitute an unstable phenotype that can have both pro-inflammatory and immune-dampening, homeostatic functions, depending on the tissue context and polarising environment. Transforming growth factor (TGF)-β in conjunction with IL-6 contribute to upregulation of transcription factor RORγT, STAT3 and T_H17 differentiation (12). T_H17 cells can produce IL-17A, IL-17F, IL-21 – which acts in an autocrine feed-forward manner to stabilise the T_H17 phenotype – and IL-22. While crucial in the immune response against parasites, T_H17 cells are also heavily implied in autoimmune diseases such as rheumatoid arthritis (RA) (13, 14), psoriasis (15, 16) and multiple sclerosis (MS) (17). It has been demonstrated that in the presence of IL-23, the T_H17 programme is skewed towards a more pro-inflammatory, granulocyte-macrophage colony-stimulating factor (GM-CSF)-producing and potentially harmful phenotype, while absence of IL-23 instead fosters a regulatory state and shifts the cytokine profile towards IL-10 and possibly IL-26 (18, 19). The balance between these two variants may be, at least in part, attributed to the target organ: in the gut and the lung, IL-17 and IL-22 have been shown to play a protective role in infectious disease as well as in tissue repair and dampening of fibrotic progression (20-22). It is possible that T_H17 responses are involved in local homeostasis in mucosal organs, while at the same time being detrimental at other sites such as the joint, skin or nervous system.
In addition to the more established subsets, T_H22 and T_H9 cells have recently been described as functionally distinct from T_H17 and T_H2 cells, respectively; however, these remain incompletely characterised.

T_{REG} cells are generally characterised by expression of transcription factor FoxP3, and a combination of surface receptors CD25, cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), CD39, CD73, programmed cell death protein-1 (PD-1), CD103, CD27 and lack of CD127 (23-31). The true definition of a regulatory phenotype, however, is based on the cell's capacity to suppress effector cells, which can occur despite lack of several of the above-mentioned markers and differences in *FOXP3* gene methylation status (32, 33). Notably, FoxP3 is also upregulated in effector cells undergoing activation (34), and can be expressed in conjunction with other transcription factors. Joint T-bet and FoxP3 expression has recently been

suggested to be involved in maintaining local tissue homeostasis (35, 36). The majority of T_{REG}s develop through high-avidity recognition of self-antigens, and subsequently act to prevent tissue damage caused by autoreactive T cells that evade negative selection in the thymus. Regulatory cells can also be induced in the periphery in response to TCR stimulation in combination with other factors such as IL-2 and TGF-β (37). T_{REG}s restrict immune activity both through secretion of anti-inflammatory cytokines such as IL-10, IL-35 and TGF-β, as well as through direct binding of inhibitory co-receptor CTLA-4 to CD80/CD86 on APCs with higher affinity compared to CD28, thereby competing out its co-stimulatory effects (38). Dysregulation of T_{REG} function is a common trait in several chronic inflammatory disorders (39).

1.7 T CELL PLASTICITY

Though still frequently used, recent advances in the field of T cell immunology and increased understanding of the concept of $CD4^+$ T cell plasticity and multifunctionality has brought into question the conventional monolithic paradigm of $CD4^+$ T cell lineage commitment and terminal differentiation into distinct subsets. Rather than a stable lineage phenotype from which a cell does not diverge for the remainder of its lifespan, a cell may over the course of an immune response express phenotypic and functional characteristics of several subsets, switching between different states depending on the tissue microenvironment, the triggering antigen and the disease course.

Given the diverse nature and uncountable number of microbial pathogens, as well as the gradual development of increasing numbers of memory cells that may cause inappropriate responses if exposed to new pathogens with similar, but not identical, characteristics, it is only reasonable to assume that $CD4^+$ T cells ought to, by design, be able to specifically tailor their responses in retort to the threat at hand. It is therefore highly plausible that the local cytokine milieu influences $CD4^+$ T cell polarisation depending on the need of the host, thus utilising the full repertoire of $CD4^+$ T cells to generate a more efficient immune response (40). Accordingly, cytokine profiles have been found to be far more flexible than formerly understood, with IFNγ being produced by either T_H1 or T_H17 cells; T_H1, T_H2, T_H17 as well as T_{REG}s being able to produce IL-10; IL-9 production by either T_H2 or T_H17 cells; complete loss of IL-17A production by T_H17 cells to become sole IFNγ producers; and simultaneous production of IL-22 and IFNγ, among others (40). Likewise, expression of master transcription factors that are believed to drive differentiation programmes is not fixed. In addition to the transient expression of

FoxP3 during effector cell activation and localised co-expression of FoxP3 and T-bet, T_{REG}s may express FoxP3 in conjunction with RORγT, despite the two being believed to constitute opposing end points of the same differentiation pathway (41, 42). Interestingly, IL-17-producing T_{REG}s are still suppressive (43, 44). Much of this instability, or flexibility, can probably be attributed to the migratory patterns of $CD4^+$ T cells, whereby irreversible commitment could confer a disadvantage under rapidly changing conditions or in diverse tissue microenvironments. A more nuanced and accurate view of T cell differentiation may therefore be that of a dynamic gradient of transcription factor expression, with $CD4^+$ T cells preferentially expressing particular transcriptional regulators but being able to adapt to shifts in the microenvironment, or throughout different phases in the life of a cell, by up- or downregulating other factors. Rather than an "on-and-off" state of transcription factor expression, a more appropriate depiction may be an array of different transcription factors, expressed at different ratios relative to one another, that ultimately govern the specialised function of $CD4^+$ T cells at a particular tissue site and moment in time (40) (Fig. 4).

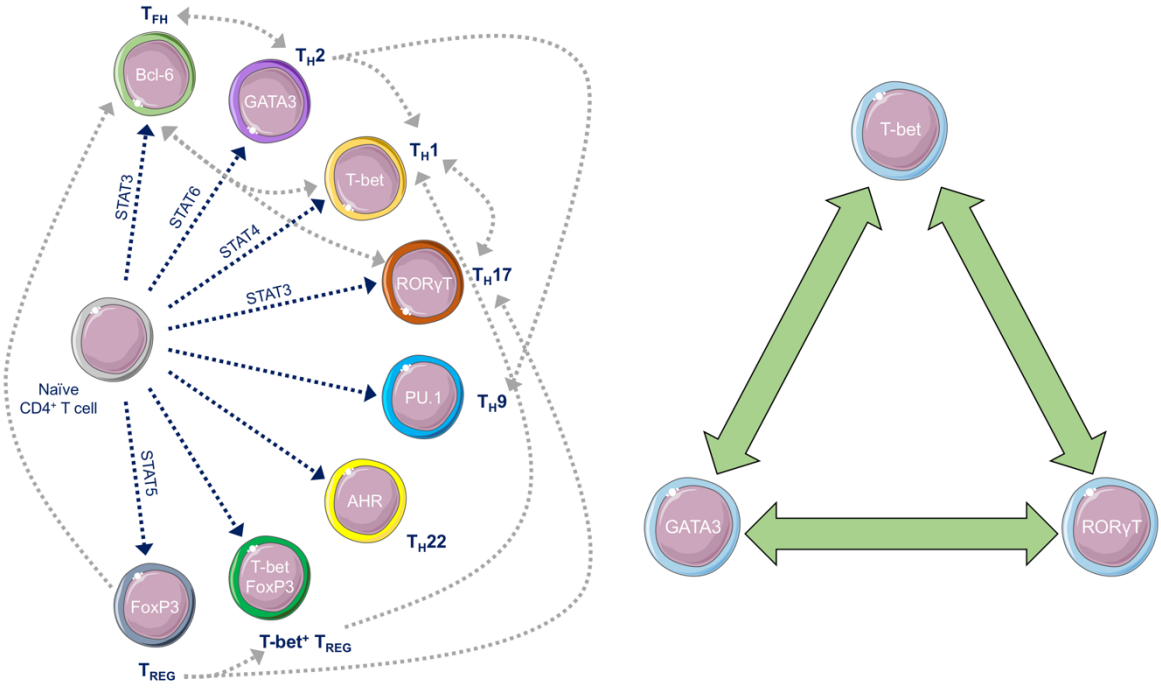

Figure 4 | $CD4^+$ T cell plasticity. Schematic outline of documented T_H subsets, along with their "master" transcriptional regulators and postulated pathways of inter-differentiation. The delicate balance of transcription factor expression believed to ultimately dictate cell fate at a certain, compartment-specific time point is here represented by green arrows. Rather than entering a state of terminal differentiation, the cell is thereby able to respond to various stimuli in its microenvironment and adapt its functionality accordingly.

1.8 ANTIBODIES

Antibodies, also termed immunoglobulins (Ig), are Y-shaped proteins that can exist either in membrane-bound form as part of the B cell receptor (BCR) complex mediating antigen recognition, or secreted, serving to neutralise and eliminate microbes and toxins. The general structure of an antibody comprises two identical heavy chains and two light chains, each chain containing a variable (V) and a constant (C) region. The combined V domains of the heavy and light chains together constitute the antigen-binding region, where, similarly to the TCR, three CDR loops act as the key site for antigen recognition. In humans, five different antibody isotypes exist, distinguished by their structure as well as their effector functions: IgM, IgD, IgG, IgA, and IgE. IgM is the first antibody produced and is secreted in the form of a pentamer with high avidity, acting to eliminate pathogens in the early stages of humoral immunity. Together with IgD, IgM constitute the BCR repertoire of naïve B cells. IgD also stimulates basophils and mast cells to produce antimicrobial factors (45). IgG is the primary isotype found in the circulation and exists in four subclasses, in humans termed IgG_1, IgG_2, IgG_3 and IgG_4, which differ in abundance and sensitivity to various antigenic sources. General functions of IgG include opsonisation of foreign antigens for phagocytosis by macrophages and neutrophils, neutralisation of microbes and free toxins, and promotion of antibody-dependent cellular cytotoxicity by NK cells. In addition, maternal IgG is able to transfer across the placenta to mediate neonatal immunity. IgG antibodies also provide feedback inhibition to B cells by binding to inhibitory receptors. IgA is found primarily in mucosal organs, e.g. the respiratory, gastrointestinal and urogenital tracts, as well as in mucous bodily fluids such as saliva, tears and breast milk, where it facilitates rapid neutralisation of toxins and microbes. Finally, IgE is an essential component of the defence against helminthic parasites, but is also responsible for mediating immediate hypersensitivity reactions by binding to allergens and triggering mast cell degranulation of histamine (1).

1.9 B LYMPHOCYTES

B cells, effector cells of the humoral branch of the adaptive immune system, develop and mature in the bone marrow, undergoing V(D)J recombination of the heavy and light BCR chains similarly to their TCR equivalents. Following successful receptor assembly, transitional B cells, recognised by a high surface expression of IgM and IgD, leave the bone marrow and migrate to the spleen to undergo the final stages of maturation. Once mature, their primary effector

function is the production of highly specific antibodies, which act to neutralise, or otherwise opsonise and target foreign antigens for destruction. Within peripheral lymphoid organs, T cells and B cells are localised in separate compartments; at homeostasis, B cells reside in distinct structures termed follicles, while T cells are dispersed outside but adjacent to the follicles (1).

1.9.1 B cell activation

As opposed to T cells, which almost exclusively react to peptide antigens, B cells recognise conformational as well as linear epitopes derived from a wide range of distinct antigenic compounds, including proteins, lipids, polysaccharides, nucleic acids and small chemicals. Protein antigens are processed by APCs and recognised by T cells, which in turn provide stimulatory signals to B cells to initiate antibody production; such antigens are termed "T cell-dependent". Non-protein antigens are able to propagate antibody responses without T cell interaction and are therefore called "T cell-independent". Similarly to T cells, clustering of membrane Ig receptors initiates a downstream biochemical signalling cascade ultimately resulting in activation of transcription factors responsible for B cell proliferation and differentiation. Signal transduction requires cross-linking of several surface Ig receptors, which occurs during binding of an aggregate of antigenic molecules, or with repeating epitopes within the same molecule, which is commonly observed in larger molecules such as polysaccharides and lipids. Non-covalent association of the BCR with the Igα and Igβ chains mediates intracellular signal transduction through the BCR complex, with a series of phosphorylation steps similar to that of TCR signalling. Importantly, B cells are able to recognise unprocessed antigens, resulting in subsequent production of antibodies with the same specificity as the reactive surface BCR. The early phase of the humoral immune response therefore generally involves secretion of soluble IgM, the antigen receptor of naïve B cells.

1.9.2 T-B cell interaction

As most protein antigens do not contain repetitive epitopes, their ability to independently stimulate B cells is limited, and generation of efficient antibody responses therefore requires interaction of T and B cells specific for the same antigen. Fortunately, B cells are highly potent professional APCs for antigens they specifically recognise. Hence, BCR recognition of a protein antigen leads to its internalisation and degradation, followed by presentation of T cell-specific epitopes on HLA class II molecules (Fig. 5).

Upon activation, B cells downregulate expression of chemokine receptor CXCR5, which serves to retain naïve cells in the follicles, and instead upregulate CCR7, enabling migration towards the border of the T and B cell zones. Conversely, $CD4^+$ T cells activated by the same antigen reduce their expression of CCR7 and instead express CXCR5, thereby causing movement towards the B cell zone. Initial T-B cell contact occurs in the extrafollicular space, where B cells present the processed antigen to T cells, ensuring antigen reactivity of T and B cells remains the same although the TCR and BCR recognise different epitopes. In return, B cells receive "T cell help" through the interaction of CD40L on the activated T cell and CD40 on the B cell. Engagement of the CD40 receptor promotes clonal expansion of B cells and differentiation into antibody-producing plasma cells. Extrafollicular plasma cells are usually short-lived, and produce antibodies of relatively low affinity. Following interaction with B cells, $CXCR5^{high}$ T_H cells can migrate into the follicles and differentiate into follicular T helper (T_{FH}) cells. T_{FH} cells may originate from either naïve T cells or previously differentiated cells of any T_H subset, in which case they will continue to produce subset-specific cytokines. In general, T_{FH} cells are commonly distinguished by their expression of PD-1 and inducible co-stimulator (ICOS), in addition to CXCR5 and CD40L, as well as transcription factor Bcl-6 and production of high levels of IL-21 (1).

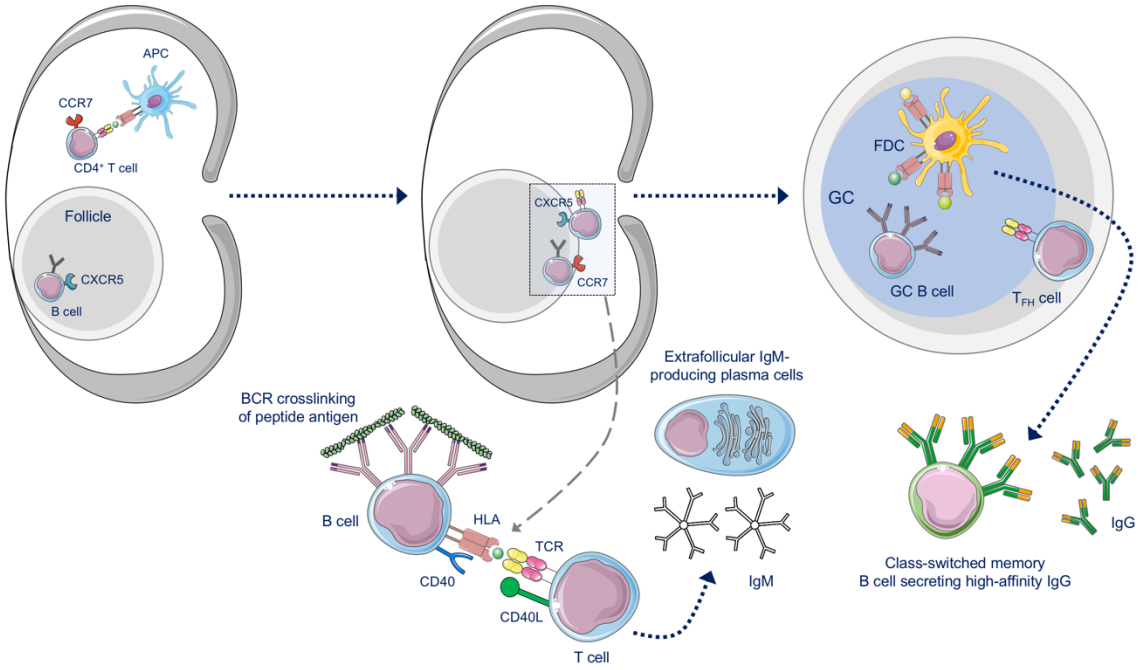

Figure 5 | T-B cell interaction. Graphic representation of the initial stages of T-B cell interaction in secondary lymphoid organs, depicting the opposing switch in chemokine receptor expression allowing for synchronised migration to the follicular border, subsequent interaction of T and B cells activated by the same antigen, and differentiation of B cells into antibody-producing plasma cells. Continuous antigen presentation by FDCs and survival signals provided by T_{FH} cells are central to the GC reaction and generation of class-switched, high-affinity B cells.

1.9.3 Class-switch recombination and affinity maturation

Activated B cells also migrate back into the follicle, where their continued interaction with T_{FH} cells initiates isotype switching and affinity maturation in a specialised structure termed the germinal centre (GC) (Fig. 5). A combination of CD40L-mediated signals and cytokines released from T_{FH} cells stimulates B cell expression of the enzyme activation-induced deaminase (AID), which drives rearrangement of the Ig heavy chain gene. Splicing of the previously recombined VDJ exon onto different constant region exons generates antibodies of different subclasses, but with the same epitope specificity, thus facilitating specialised effector functions that are ideally suited to the nature of the antigen. Moreover, extensive somatic hypermutation orchestrated by AID introduces point mutations in the V region, and particularly the hypervariable CDR3 segment primarily involved in antigen binding, in order to increase antibody affinity. Continuous antigen presentation by specialised follicular dendritic cells (FDCs) in the GC enables antigen-driven selection of B cells with the highest-affinity BCR. These cells are provided survival signals and T_{FH} help, promoting further mutagenesis and production of new antibodies. GC B cells may enter the circulation as mature, long-lived plasma cells, which continue to produce high-affinity antibodies long after antigen clearance and provide immediate protection upon re-exposure. Other isotype-switched, affinity-mature B cells persist in the form of memory cells, which reside in tissues and monitor the circulation, responding rapidly to reintroduction of the antigen with clonal expansion and production of already class-switched high-affinity antibodies (1). Although the mechanisms that govern differentiation into either plasma or memory cells are not fully understood, the duration of T-B cell interaction (46, 47), the time point of the GC reaction when cells are generated (48), regulation of transcription factor Bcl-6 by CD40 and IL-21 signalling (49, 50) and affinity for the antigen (51) have all been proposed to influence B cell fate.

1.10 LYMPHOCYTE SELECTION, TOLERANCE AND AUTOIMMUNITY

During maturation, both T and B cells undergo a sequential selection process, the purpose of which is to ensure functionality of the antigen receptors as well as deletion of potentially harmful, self-reactive cells. Initial selection is provided during TCR and BCR generation; failure to generate a functional receptor results in apoptosis. Once the whole receptor has been assembled on the cell surface, its ability to recognise ubiquitous self-antigens, such as abundant blood proteins or membrane molecules, will determine cell fate. A high-affinity binding to the HLA-

antigen complex results in death by negative selection, as these cells may be activated without the need for second signals, and could propagate destructive autoreactive responses. Conversely, lack of recognition, which would render the cell incapable of mounting a response to harmful agents, leads to death by neglect, as the cell does not receive the appropriate survival signals from the APC. Positive selection occurs when the antigen is recognised with low affinity, the intracellular signalling cascade being triggered by the aid of co-receptors and co-stimulatory molecules on the APC. Specifically, immature T cells express both co-receptors CD4 and CD8, and recognition of an antigenic peptide in complex with either HLA class I or class II results in downregulation of the unbound co-receptor and thereby commitment to either the $CD4^+$ T_H or the $CD8^+$ CTL lineage (1).

Immunological tolerance refers to the lack of response elicited by lymphocytes exposed to certain antigens, most commonly harmless foreign agents and self-antigens. Central tolerance is mediated through negative selection in the lymphoid organs, as well as generation of T_{REG}s that act to restrict self-reactive lymphocyte responses in the periphery through secretion of immune-dampening cytokines and expression of co-inhibitory receptors. Peripheral tolerance also comprises induction of anergy, which occurs when an antigen is recognised without proper co-stimulatory signals and the lymphocytes are rendered functionally inactive. Autoreactive cells can also be deleted by triggering of apoptosis, either through release of pro-apoptotic signals or expression of the death receptor-ligand pair Fas-FasL in response to recognition of self-antigens (1). Importantly, however, estimates have shown approximately 20% of circulating B cells to be autoreactive without causing disease (52). Conversely, as tissue-specific or intracellular antigens are normally not presented in secondary lymphoid organs, lymphocytes recognising these antigens may not be deleted by negative selection and are thus able to undergo clonal expansion unhindered when confronted with release of intracellular components during necrosis, or tissue-specific factors secreted during trauma.

Failure of tolerance mechanisms, which commonly occurs due to a combination of genetic and environmental factors, can result in autoimmunity. HLA genes are the most commonly associated with heritable predisposition, and different HLA alleles and haplotypes have been shown to influence susceptibility for or protection against the development of certain immune-mediated and autoimmune diseases. For example, *HLA-DRB1*15* (DR15 haplotype) is tightly linked to a predisposition

to develop MS (53, 54), and *HLA-DRB1*04* (DR4) to RA (55). *HLA-DQB1*02* (DQ2.5) confers increased risk of coeliac disease, while its homologue DQ2.2 reduces the risk of the same condition (56). In addition, polymorphisms in genes associated with lymphocyte regulation or activation, such as CTLA-4, CD25 (the IL-2 receptor α chain), the IL-23 receptor (IL-23R) and protein tyrosine phosphatase N22 (PTPN22) have also been implicated in several multifactorial autoimmune diseases (1). Furthermore, autoreactive lymphocytes can be activated during an infection, if the presented epitopes closely resemble the specificity of the TCR or BCR. Other potential risk factors for development of autoimmunity include exposure to environmental agents (57), gender (58), and smoking (59).

1.11 THE RESPIRATORY SYSTEM

1.11.1 Anatomy of the airways

The lungs are located in the thoracic cavity, protected by the rib cage and separated from the gastrointestinal system by the diaphragm, which is controlled by the sympathetic nervous system and the main muscle involved in breathing. The left lung, which shares space with the heart, is slightly smaller and consists of two lobes, while the right lung comprises three. Each lung is enclosed within a pleural sac, containing a serous fluid that minimises friction during breathing. The respiratory tract can be divided into the upper, comprising the nasal cavity, pharynx and larynx, and lower compartment, including the trachea, bronchial tree and alveoli. Following entry through the nose and mouth, air travels through the trachea to the bifurcation between the right and left primary bronchi. These larger airways divide into secondary (lobar) and tertiary (segmental) bronchi, gradually branching into smaller bronchioles. Eventually, the terminal bronchioles cross over into the alveolar ducts, marking the transition from the conducting zone to the respiratory zone and connecting to the specialised structures of the alveolar sacs where gas exchange occurs. Between 300 and 500 million alveoli, each surrounded by a fine lattice of pulmonary capillaries facilitating transfer of oxygen to the blood and carbon dioxide back to the lungs for discharge, together provide a total pulmonary surface area of approximately 120 m^2 (60).

1.11.2 Pulmonary immunology

The airway epithelium is the first line of defence against harmful airborne agents, including microbial pathogens, toxins and pollutants. Continuous epithelia along the interface to the external environment provide steric hindrance for entry of microorganisms, as well chemical interference through production of anti-

bacterial defensins and cathelicidins. In addition to its physical barrier, ciliated cells and mucous-producing goblet cells along the epithelial lining enable efficient clearance of foreign particles. Airway epithelial cells also act as important triggers of innate and adaptive immune mechanisms in the lung through production of antimicrobial substances as well as pro-inflammatory cytokines and chemokines (61, 62). Early immune responses are also mediated by intraepithelial T lymphocytes, which express γδ TCRs of limited diversity. In contrast to traditional αβ T cells, γδ T cells do not require antigen processing and presentation by APCs and are able to recognise microbial lipids as well as general danger signals, e.g. heat-shock proteins. However, a certain degree of receptor rearrangement has been observed, as well as development of a memory phenotype, presumably in response to frequently encountered microbial threats (1).

Alveolar macrophages are resident phagocytic cells constituting 90-95% of all respiratory leukocytes in a healthy individual; in contrast, lymphocytes account for 1-10% and neutrophils roughly 1% (63). Apart from phagocytosis of harmful particles, macrophages are responsible for release of reactive oxygen species (ROS), nitric oxide (NO) and proteolytic enzymes that can directly kill invading pathogens (64), as well as antigen presentation to T cells. In addition, DCs residing beneath the airway epithelium migrate from the mucosa to the thoracic lymph nodes to present antigen and prime T cell responses (65). Unique to mucosal organs are discrete collections of lymphocytes and APCs with a similar organisation to that observed in lymph nodes. At any given time, at least a quarter of the body's lymphocytes reside in the mucosal tissues and skin, most of which are memory cells. Mucosal tissues also comprise a specific subset of B cells, termed B-1 cells, which are self-renewing and able to secrete natural antibodies, i.e. antibodies produced even in absence of encountered pathogens, in response to non-protein antigens without requirement for T cell help, as well as to generate memory responses (66). The primary antibody type produced in mucosal lymphoid organs is IgA, which can be transported across the epithelium, bind to and neutralise microbes present in the lumen of the respiratory tract. Cytokines inducing class-switching of B cells to IgA, most prominently TGF-β, are constantly produced at high levels in mucosa-associated lymphoid organs, and IgA-producing B cells are predisposed to recirculate to mucosal tissues and home to specific sub-epithelial sites (1).

Insufficient or exaggerated immune responses in the lung can lead to pathological conditions, such as asthma, chronic obstructive pulmonary disease (COPD), and interstitial lung diseases (ILDs), which include, among others, idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonia, chronic beryllium disease (CBD), silicosis and sarcoidosis. Though the triggering factor may vary, ILDs (also termed diffuse parenchymal lung diseases) share distinct clinical features of diffuse lung infiltrates on chest radiography and distortion of gas exchange, resulting in restriction of respiratory volume and impaired oxygenation (67, 68).

1.12 SARCOIDOSIS

1.12.1 Clinical characteristics

Sarcoidosis is a granulomatous multisystem disorder of unknown aetiology. The disease primarily manifests in the lungs, which are affected in approximately 90% of cases, but can also target other organs such as the eyes, spleen, skin, liver, kidney, heart and central nervous system (CNS) (69, 70). It mainly affects young adults between 30-50 years of age, but may arise in adults of all ages; especially for women, a second peak of incidence can be observed around 60 years of age (71). Globally, the disease is most prevalent in Northern European countries, where disease is usually resolving, and in African-Americans, who generally experience a more severe disease and are prone to develop chronicity (72). The incidence in Sweden is approximately 1200 new cases yearly, and more than half of all patients develop a disease with more than two years' duration. While the main clinical symptoms are a non-productive cough and dyspnoea, fatigue and arthralgia are common features with major influence on the patient's quality of everyday life (73, 74). For patients with chronic disease, pulmonary fibrosis, cardiac and CNS involvement are the major causes of sarcoidosis-associated mortality (69).

1.12.2 Immunopathogenesis and diagnosis

Histologically, the characteristic finding in sarcoidosis is the presence of non-caseating granulomas in the affected organs (Fig. 6). These aggregates of highly differentiated immune cells develop upon accumulation of alveolar macrophages and DCs at sites of inflammation and an ensuing inability to completely eliminate the antigenic source, which resists degradation either due to size, insolubility or inherent anti-phagocytic properties. Instead, under the influence of abundant inflammatory signals, activated macrophages and DCs fuse to form multinucleated giant cells (MGCs) (75), which are surrounded by activated T cells to which antigen

is presented (76). Encapsulation of the antigen by deregulated immune cells prevents its dissemination, while the failure of antigen clearance results in persistence of granulomas, in severe cases leading to necrosis and fibrotic scarring of associated tissue. The sarcoid granuloma consists of two segments: the core region, comprising tight clusters of macrophages, epithelioid cells and a few MGCs, and the surrounding crust, containing high numbers of T cells and few B cells (77). Although not typically observed within sarcoid granulomas, higher numbers of neutrophils have been detected in patient lungs compared to healthy controls (78), and especially in patients with more severe disease (79). Moreover, impaired oxidative phosphorylation of mitochondrial proteins is believed to contribute to ROS production, inflammatory signalling and tissue damage in chronic sarcoidosis (80). DCs from sarcoidosis patients also produce higher levels of TNF-α compared to healthy individuals, suggesting a feed-forward loop driving DC maturation, migration and granuloma formation (81).

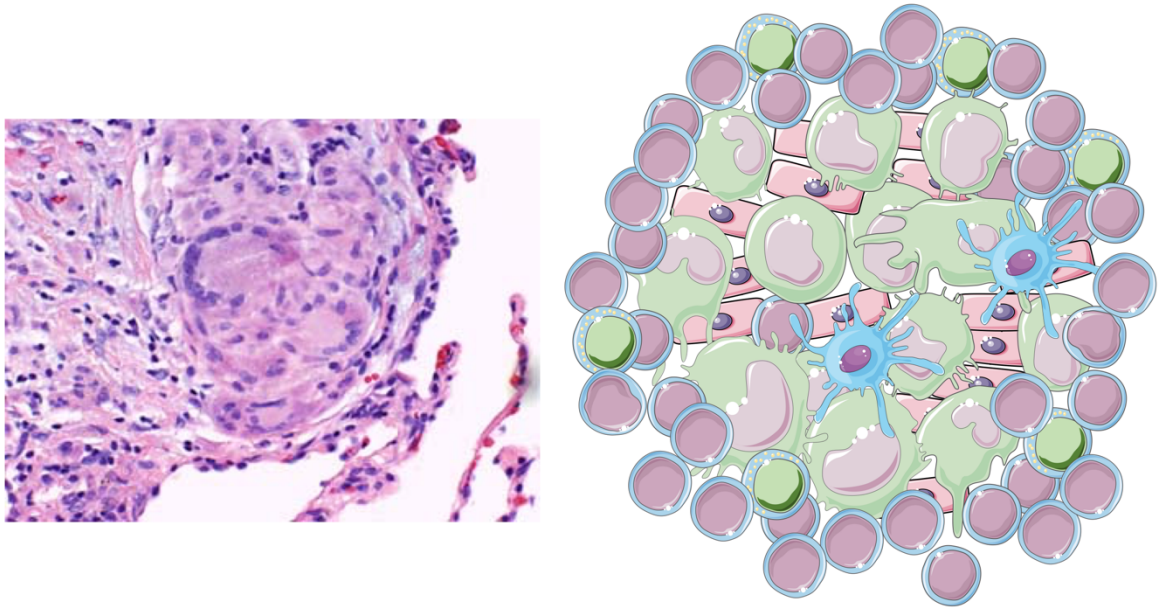

Figure 6 | Structure of the sarcoid granuloma. Histological image of a classically well-structured non-caseating granuloma, accompanied by a graphic representation of its components. Most prominently, the sarcoid granuloma comprises a core of macrophages and DCs, occasionally fused to MGCs, surrounded by an outer crust of lymphocytes, the majority of which are $CD4^+$ T cells, interspersed with $CD8^+$ T cells and a few B cells.

Lymphocyte involvement is dominated by T cells and particularly $CD4^+$ T_H cells, which are essential contributors to granuloma formation, maintenance and overall disease outcome. IL-12 and IL-18 secreted by macrophages and DCs at the inflammatory site drive differentiation of T cells, which in turn secrete high levels of IFNγ and IL-2, propagating both macrophage activation and T cell survival (76). Release of ligands for chemokine receptor CXCR3, most notably CXCL10, by macrophages further ensures migration of T cells to the granulomatous region

(82). In addition, IL-17A-producing memory T cells are found within and in the vicinity of sarcoid granulomas (83, 84), and both IFNγ and IL-17A are believed to be central to the process of granuloma formation (85-87).

On a cellular level, one of the hallmark signs of sarcoidosis and a major diagnostic criterion is a pronounced lymphocytosis (>25%) and an elevated CD4/CD8 T cell ratio (>3.5) in bronchoalveolar lavage fluid (BALF) (88), and repeated studies have firmly established sarcoidosis as a primarily $CD4^+$ T cell-driven disease (69, 89). Additional diagnostic parameters include characteristic findings on chest radiography (Table 1) and lung function tests, as well as measurement of blood biomarkers, most prominently elevated levels of serum angiotensin-converting enzyme (ACE) and free calcium, which associate with macrophage activation and have been postulated to reflect an increase in granulomatous activity (90). A negative tuberculin (PPD) test, as well as exclusion of differential reasons for granuloma formation, such as malignancy, infectious disease or systemic autoimmunity, are also required for establishing the diagnosis.

Chest radiographic stage	Characteristic findings
0	Normal findings
I	BHL without pulmonary infiltrates
II	BHL with pulmonary infiltrates
III	Only pulmonary infiltrates without BHL
IV	Signs of fibrosis and volume reduction

Table 1 | Clinical staging in sarcoidosis according to findings on chest radiography.

1.12.3 Clinical and molecular basis of disease phenotypes

Sarcoidosis appears in two forms, which are mainly distinguished based on clinical criteria; the underlying immunological mechanism(s) largely remain unknown. Löfgren's syndrome (LS), first described in 1946 by Swedish pulmonologist Sven Löfgren (91), presents with an acute onset, usually fever, active inflammation and characteristic clinical symptoms such as bilateral hilar lymphadenopathy (BHL) (Fig. 7), erythema nodosum and/or ankle arthritis. Women more frequently present with erythema nodosum, while men commonly develop bilateral ankle arthritis (92). In Sweden, approximately one third of all sarcoidosis patients are diagnosed with LS (93, 94). "Non-LS" patients show a slower onset and are more heterogeneous in disease presentation, but commonly suffer from fatigue, low-

grade fever and weight loss. While LS patients usually experience a more rapid and spontaneous disease resolution and have a good prognosis, non-LS patients are at a higher risk of developing chronic disease, of which 20-25% eventually progress to pulmonary fibrosis. Mortality rates range between 1-5%, usually due to severe fibrosis and/or myocardial or CNS engagement (69). Due to its distinct clinical characteristics, LS has even been suggested to be considered as a separate disease (93). Recent genetic studies have revealed distinct differences between LS and non-LS, with LS patients being genetically more homogenous, and exhibiting gene associations not present in non-LS, and *vice versa* (95). LS is closely linked to presence of specific HLA allele *DRB1*03*, which appears to be a strong prognostic factor in sarcoidosis. Of HLA-DRB1*03^{+} LS patients, 95% resolve spontaneously within two years; for HLA-DRB1*03^{-} LS patients, this number plunges to 50% (93). Other HLA-DR alleles have also been demonstrated to influence disease outcome; most notably, *HLA-DRB1*14* and *HLA-DRB1*15* appear to associate with chronicity (96-98), while *HLA-DRB1*04* is overrepresented among patients with ocular involvement (99). In addition to HLA genes, various polymorphisms in the butyrophilin-like 2 (*BTNL2*) gene have been shown to influence development of LS and non-LS, respectively (100). BTNL-2 is a co-inhibitory receptor distinct from the CD28-CTLA-4 axis, acting to negatively regulate T cell activation by promoting *de novo* FoxP3 expression (101). Moreover, polymorphisms in chemokine receptor genes *CCR2* and *CCR5* have, independently of *HLA-DRB1*03* (102, 103), been associated especially with LS, and in the case of *CCR5*, specifically in females (104). Other prominent genetic variants linked to development of either LS or non-LS disease include the major histocompatibility complex 2 transactivators (*MHC2TA*) (105), macrophage migration inhibitory factor (*MIF*) (106) and isoforms of TGF-β (107).

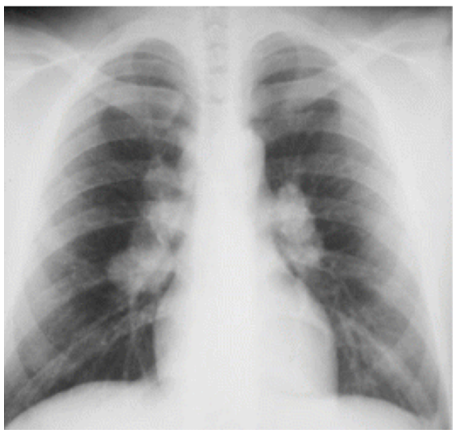

Figure 7 | **Bilateral hilar lymphadenopathy on chest radiograph.** A representative image of the bilateral enlargement of hilar lymph nodes commonly observed in sarcoidosis, and most notably in LS, as visualised by standard chest radiography.

1.12.4 Disease aetiology

The causative agent(s) in sarcoidosis have yet to be identified. Over the years, postulated causes have included infectious agents (108), e.g. *Mycobacterium tuberculosis* (109, 110) or *Propionibacterium acnes* (111-113), exposure to environmental irritants such as dust particles from construction sites (114), protein misfolding (115), or, most recently, autoimmunity (116, 117).

Sarcoidosis has been shown to be transmissible to naïve transplant recipients from donors not known or suspected to have active disease (118), reminiscent of involvement of an infectious agent. The strive for HLA compatibility during transplantation should, however, be accounted for, as an environmental or self-antigen readily available in the inflammatory environment of the donor could theoretically be recognised also by a recipient with a shared HLA background.

The similarity of sarcoidosis pathology to that of tuberculosis, most prominently characterised by granuloma formation in the lungs, initially suggested a common origin. Myco- and propionibacterial DNA and protein have been identified in sarcoid granulomas, and several studies show reactivity of lung-derived T cells to mycobacterial proteins (109), most frequently mycobacterial catalase-peroxidase (mKatG), the 6 kDa early secretory antigenic target (ESAT-6) and heat-shock proteins (119, 120). Opinions differ on the exact disease mechanism, but it has been suggested that sarcoidosis is caused by immune reactivity towards incompletely degraded bacterial antigens (121). However, as presence of bacterial DNA (122) and protein (110) has been reported in approximately 50% of investigated sarcoidosis patients, and as live mycobacteria have never been identified in sarcoid lungs, these findings cannot account for all patients. Moreover, mechanistic studies of *M. tuberculosis* infection show that the granulomatous environment itself attracts infected cells that may contribute to augmented pathogenesis and prolonged immune responses, which could account for the presence of bacterial remnants without clinical signs of a previous infection.

While a seasonal variation in disease onset (123), particularly for LS, points to involvement of an infectious agent, another explanation could be the influence of an environmentally-derived triggering factor, the accessibility of which fluctuates with the seasons. Fittingly, sarcoidosis has been associated with exposure to environmental irritants such as tree pollen, inorganic particles and mouldy

environments (124, 125). Moreover, an increased incidence of sarcoidosis-like disease has been observed in construction workers (126), military personnel and firemen (69), most recently in the aftermath of the World Trade Center catastrophe in 2001 (114, 127-129). However, such exposure also appears to associate with other, unrelated lung diseases (130).

Serum amyloid A (SAA) proteins are secreted from the liver during the acute phase of inflammation, serving to recruit immune cells to the site of injury and mediate induction of enzymes that degrade the extracellular matrix. Macrophages are a major source of SAAs in inflammatory conditions, and elevated levels of SAA have been reported in RA, diabetes type II and Crohn's disease (131), as well as in sarcoidosis (132, 133). Interestingly, expression of SAA in sarcoid granulomas appears to be distinct from that of other granulomatous disorders, including CBD, Crohn's disease, Wegener's granulomatosis, and tuberculosis. It has been hypothesised that SAA is generated as part of the acute phase reaction following an infection, and that misfolded and/or aggregated protein products propagate further release and accumulation of SAA in the granuloma. Inability to clear SAA and trapped antigens, partly dependent on genetic and/or epigenetic factors, would thereby lead to chronic inflammation and fibrosis (119, 134). However, SAA induction is a rapid, unspecific response, with elevated levels being detected within hours of exposure to often relatively trivial inflammatory stimuli, and might therefore be a general sign of an ongoing inflammatory reaction rather than a disease-specific trigger in itself. Nonetheless, its value as a prognostic marker for chronic disease as opposed to remission ought to be further explored.

The concept of sarcoidosis as an autoimmune disease is supported by the strong HLA connection in LS patients, and the higher prevalence of disease in first-degree relatives (135). Moreover, a markedly higher risk of developing disease has been documented in monozygotic compared to dizygotic twins (136), suggestive of an imperative hereditary component. In addition, these patients are generally young, previously healthy, and non-smokers (94). If this is the case, however, sarcoidosis would be one of very few self-limiting autoimmune conditions, as the disease, especially in LS patients, usually resolves spontaneously. Nevertheless, it has been shown that in a small group of patients (<5%), the disease can relapse later in life (137, 138), though whether this is to be regarded as re-infection or an autoimmune flare remains unknown. Notably, in HIV^+ patients receiving highly active anti-retroviral therapy (HAART), sarcoidosis has been observed to appear

with regained $CD4^+$ T cell counts >200 cells/mm^3 (139, 140), which is the opposite scenario from what would be expected in an infectious disease. Furthermore, sarcoidosis-like disease has been reported in patients administered IFN treatment for malignancy, hepatitis C infection or immune-mediated disorders (141-143). Patients treated with anti-CTLA-4 or anti-PD-1 (e.g. due to metastatic melanoma) are also at higher risk of developing sarcoidosis (144-150), possibly due to impaired T_{REG}-mediated responses, again implicating immune hyperactivity rather than deficiency in disease pathogenesis.

In 1941, Norwegian pathologist Morten Ansgar Kveim first reported the diagnostic use of a skin test based on intradermal injection of a pasteurised saline suspension of sarcoid lymph node tissue (151). Over the following weeks, patients with active sarcoidosis, but not healthy controls, would develop granulomatous structures in the skin at the site of injection, which would be verified as sarcoid tissue upon histological examination (152, 153). The method was commercialised by American physician Louis Siltzbach in 1954, then using spleen tissue. Despite being discontinued in 1996 due to risk of transferred infections, the use of such a mixture of undefined, granuloma-derived proteins for diagnostic purposes suggests reactivity to one or several of the contents of the Kveim reagent to be central to the disease process (154-156). One such recently identified component is vimentin, a type III intermediate filament protein expressed in mesenchymal cells and an important constituent of the cellular cytoskeleton (157). Previous studies utilising mass spectrometry have, on separate occasions, identified a vimentin-derived peptide as bound to HLA-DR on alveolar macrophages in BALF of HLA-DRB1*03$^+$ sarcoidosis patients (116, 117, 158). Intriguingly, the *HLA-DRB1*03* allele has also been shown to influence the outcome of autoimmune disease systemic lupus erythematosus (SLE) (159), as well as thus far unresolved conditions such as Langerhans cell histiocytosis (160) and idiopathic inflammatory myopathies (161, 162). In SLE, elevated levels of anti-vimentin antibodies have been found in patients with the severe kidney manifestation tubulointerstitial nephritis (163), implicating vimentin in the pathogenesis of several autoimmune disorders. Following citrullination, a process commonly induced by smoking, vimentin also acts as a target in RA (164, 165). Antibodies against citrullinated vimentin can be found in the lungs prior to clinical onset of disease (166), suggesting that disease-initiating events in autoimmune conditions may occur in the lungs regardless of the target organ from which clinical symptoms are observed. In contrast to RA, smoking is not considered a risk factor for sarcoidosis

and even shows a negative correlation with disease development (124), which could possibly be explained by differences in vimentin reactivity: in one case the modified form of the protein is recognised, in the other, the native form.

It is also possible that the true cause of the disease stems from a combination of the above-mentioned factors, as in the case of transient autoimmunity (167, 168). Alternatively, dual reactivity to several antigens of different origin can arise due to molecular mimicry. TCR recognition of a microbial antigen followed by cross-reactivity of activated T cells to a structurally similar self-peptide can result in autoimmune responses, which abate as the pathogen is cleared (169). A third possibility is that of an autoimmune reaction following allergic hypersensitivity (170), for example to certain metals (171) or inorganic compounds. Exposure to silica, which is abundant in nature, as well as an integral component of several industrial products such as glass, ceramics and cement (172), has been associated with silicosis (173), which is usually a progressive, fibrotic condition, but also with RA, SLE and sarcoidosis (174). Modified silica particles, e.g. in crystalline format (175), could promote macrophage activation, antigen presentation and subsequent responsiveness not only to silica but also to cellular components released by damaged macrophages. Intriguingly, recent years have seen the successful exploration of CBD, another granulomatous lung disorder with a strong HLA association (in this case to HLA-DP2) and notable similarities to sarcoidosis in terms of disease presentation and histological picture. It was found that all affected patients had been previously exposed to beryllium, allowing Be^{2+} ions to interfere with TCR recognition of HLA complexes presenting self-peptides in the lung. Upon Be^{2+} binding, T cell epitopes would be altered, subsequently causing recognition of a self-peptide (in this case plexins derived from the pulmonary epithelium) and initiation of immune responses (170, 176, 177). In contrast to sarcoidosis, however, CBD patients generally experience a chronic disease, as beryllium is not readily degraded or eradicated once it has entered the lung. The association of the Be^{2+} ion with the peptide-binding cleft of the HLA molecule also stabilises the TCR-HLA complex, ensuring continuous antigen presentation and presence of a stable, autoreactive $CD4^+$ T cell pool in the lung (170, 178, 179).

Similar to CBD, where populations of clonal $CD4^+$ T cells are observed in BALF, $CD4^+$ T cells expressing the TCR α chain variable segment Vα2.3 have previously been found to accumulate in the lungs of HLA-DRB1*03^+ sarcoidosis patients. These expansions of TCR-specific cells are found exclusively in BALF of HLA-

DRB1*03$^+$ patients with active disease, and not in HLA-DRB1*03$^+$ healthy individuals or patients with other pulmonary disorders (180). Neither are they present in regional lymph nodes, nor peripheral blood (181), and Vα2.3$^+$ T cell counts decrease upon clinical resolution (182). Moreover, in contrast to CBD, the higher the level of Vα2.3 cells at the time of lavage, the more rapid the resolution and better the prognosis (183). More recently, an accumulation of CD4$^+$ T cells expressing the Vβ22 TCR chain segment was also identified in BALF of HLA-DRB1*03$^+$ patients (184), which prompted further investigation of HLA-DRB1*03, the Vα2.3 and Vβ22 TCR segments in combination, and the proposed influence of vimentin peptides on sarcoid inflammation, the results of which are presented in this thesis.

1.12.5 CD4$^+$ T cell subsets in sarcoidosis

Overall, T cell proliferation and cytokine production is much more pronounced in the lungs than in peripheral blood, suggesting the immune reaction to be, although potentially systemic, primarily targeting an inhaled or lung-inherent antigen (181, 185). In the lungs, the vast majority of CD4$^+$ T cells are defined as effector memory T cells, characterised among others by expression of CD45RO, loss of CD27 (which occurs during prolonged antigen stimulation and T cell differentiation) (186) and elevated expression of CD69 (a marker of tissue-resident memory cells). Notably, CD69$^+$ T cells have also been postulated to be involved in autoimmune lung diseases (187).

Sarcoidosis has traditionally been considered a T_H1-driven disease, due to the high levels of IFNγ, IL-2 and TNF-α produced by BALF T cells and alveolar macrophages (134, 188), presence of T_H1-skewing cytokines IL-12 and IL-18 (185, 189, 190), as well as the high T cell expression of chemokine receptors CXCR3 and CCR5 (191). In contrast, T_H2-associated patterns of IL-4 and IL-5 production and surface expression of CCR4 are reduced in sarcoid lungs (188). However, several studies have reported involvement of IL-17 in the disease process (83, 84, 192, 193). IL-17 responses and genes involved in the T_H17 and T_H22 pathways also appear to act primarily in LS, further highlighting the importance of distinguishing between the two disease forms, both at a clinical and a molecular level (95). The role of T_H17 and so-called T_H1/T_H17 "hybrid" cells in autoimmune conditions such as RA, SLE, MS and psoriasis (13-17), where they are believed to be of pathogenic nature, in contrast to their potentially protective properties in mucosal organs, is a matter under current investigation. In sarcoidosis, IFNγ$^+$IL-17A$^+$ double-producing cells

have been implicated as the main drivers of inflammation, especially if transitioning to an IFNγ-single-producing phenotype (193), and are further recognised as mediators of glucocorticoid-refractory disease (194). However, lack of a clear consensus on how to characterise these cells in the lung complicates understanding of their functionality. It also remains to be determined whether the granulomatous environment in itself may promote T cell plasticity (195). Interestingly, IFNγ has also been shown to stimulate production of B cell-activating factor (BAFF) by monocytes (196). Elevated serum levels of BAFF have been reported in sarcoidosis patients (196, 197), and have been shown to affect T_H17 differentiation *in vitro* (198), suggesting potential B cell involvement in influencing T cell differentiation under inflammatory conditions.

T_{REG}s in sarcoidosis have also been the subject of contradictory reports, with either a reduction of $FoxP3^+$ $CD4^+$ T cells (199, 200) or an increase in numbers, but with defective functional capacity, being observed. The latter has interchangeably been measured in terms of e.g. IL-10 production (201), expression of CTLA-4 (145), survival (202) or potency in suppression of effector cell proliferation and cytokine secretion (202-205). As with effector cells, the interpretation of these results is complicated by the fact that different studies use different markers to define a particular cell subset. Especially in a tissue compartment such as the lung, the phenotype of several cells may differ significantly from that of the same subset in blood. It is also plausible that both regulatory and effector cells in tissues express specific markers that optimise activity within a certain microenvironment, potentially leading to their misclassification. Regardless of cell phenotype, increased levels of IL-10 have been observed in sarcoidosis, and especially in LS patients (201), suggesting at least parts of the disease process to involve features of immune regulation. As previously stated, inhibitory receptors CTLA-4, PD-1 and BTNL-2 are essential to T_{REG} function and their dysregulation in sarcoidosis has repeatedly been suggested to contribute to disease pathogenesis (195). The closely linked developmental programmes of T_H17 cells and T_{REG}s also implicate a switch from anti-inflammatory T_{REG}s to pro-inflammatory T_H17 cells under conditions of receptor blockade (150), which impairs T_{REG}-mediated suppression. In patients with CBD, antigen persistence has been observed to reduce the requirement for CD28 signalling in response to TCR engagement, eventually resulting in dysfunctional CTLA-4 and PD-1 pathways (206, 207), which could potentially parallel the situation in chronic sarcoidosis.

1.12.6 Current and future treatment opportunities

There is currently no efficient treatment for sarcoidosis, and a majority of patients, especially with LS, remain untreated, though oral corticosteroids alone and/or in combination with methotrexate (208) or azathioprine (209) are generally prescribed in patients with severe pulmonary dysfunction and persistent infiltrates (88, 210). In general, the current therapeutic strategy is to reduce the immediate and potential long-term negative impact of disease on everyday quality of life, as well as to improve the most persistent and disabling symptoms, particularly fatigue, pain, cognitive impairment, depression and small fibre neuropathies (70). Corticosteroids constitute the first-line treatment and are usually the most effective in terms of rapid symptomatic amelioration (211). However, long-term treatment is contraindicated due to the sometimes severe side effects, including among others weight gain, diabetes, hypertension, muscular atrophy, osteoporosis, depression and infections (212, 213). Low-dose immunosuppressive and/or -modulatory drugs, most prominently methotrexate or azathioprine, are recommended as second-line treatment in steroid-refractory disease, or as a "steroid-sparing" agent when prolonged high-dosage treatment is predicted to be required (214, 215). Up to 60-80% of cases of steroid-resistant sarcoidosis have been found to respond to methotrexate (216), which has proven particularly efficient for management of ocular and neurological manifestations (217, 218). Azathioprine has been similarly beneficial in facilitating corticosteroid reduction, as well as improving lung function parameters (209). In addition, cyclosporine has been tried as a means of restricting T cell activity and cytokine production (219). As a third-line treatment, TNF-α inhibitors, most commonly infliximab, are used for refractory sarcoidosis not responding to conventional immunosuppressive drugs, due to their role in prevention of initiation and propagation of granuloma formation (220). The efficacy of these treatment strategies is, however, highly patient-specific, and there is an evident call for superior therapeutic options (210, 221). Essentially, more individualised and targeted treatment is required to achieve complete disease resolution (if possible). Current research therefore strives to identify the disease-initiating antigen(s) in order to apply this knowledge with the aim of improving diagnostic, prognostic, therapeutic and potentially preventive measures.

2 AIMS

The two parallel, but intertwining, aims of this thesis was to conduct a detailed investigation of $CD4^+$ T cell-mediated pulmonary inflammation in clinically distinct phenotypes of sarcoidosis, and, with the aid of known TCR specificity, to identify disease-specific antigens driving such inflammation.

For the separate studies, specific aims were:

Paper I
To investigate expression of the newly identified TCR V segment Vβ22 in conjunction with Vα2.3 on lung $CD4^+$ T cells, and to evaluate the differentiation status and clonality of these cells as an indication of their possible role in antigen recognition.

Paper II
To identify factors related to T_H1- and T_H17-mediated inflammation, respectively, as well as T cell regulation, in LS and non-LS sarcoidosis, and the possible influence of T cell polarisation on clinical outcome.

Paper III
To expand on the findings in Paper II by conducting an unbiased study on $CD4^+$ T cells in the lungs of LS and non-LS patients, striving to identify novel immunological pathways that separate the two disease conditions.

Paper IV
To delve deeper into the results of Paper I by isolation and sequencing of the entire αβ TCR of sarcoidosis patients, specifically striving to elucidate consensus CDR3 motifs in HLA-DRB1*03^+ patients to be used for future antigen discovery studies.

Paper V
To complement the T cell findings of Paper I with a B cell perspective, scrutinising antibody responses to vimentin in sarcoidosis patients and T-B cell interaction in the inflamed lung.

3 COMMENTS ON METHODOLOGY

The experimental procedures underlying the work presented in this thesis are described in detail in the respective papers. This section serves as a general summary of the methodology used and its purposes, as well as limitations and pitfalls of the different techniques.

3.1 STUDY SUBJECTS

Patients with suspected sarcoidosis were committed to the Lung and Allergy Clinic at Karolinska University Hospital, Solna, Stockholm, Sweden, for primary diagnostic investigation in accordance with criteria established by the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG), American Thoracic Society (ATS) and European Respiratory Society (ERS) (88). Specifically, these included findings at bronchoscopy with bronchoalveolar lavage (BAL) including an elevated CD4/CD8 ratio, and if required, biopsies positive for granulomatous tissue, as well as exclusion of other diagnoses (primarily malignancy and infections). For all studies, patients with LS and non-LS sarcoidosis were considered separately. LS was defined as an acute onset, usually with fever, chest radiographic findings of BHL, with or without pulmonary infiltrates, and presence of erythema nodosum and/or bilateral ankle arthritis. Non-LS patients presented with varying symptomatic manifestations and severity, but commonly demonstrated an insidious onset, fatigue, dry cough and parenchymal changes. Unless otherwise specified, patients were untreated at the time of investigation. Healthy controls had normal chest radiography, no documented current infections and were not administered any medical treatment. All patients and healthy volunteers were HLA typed according to previously established protocols (222). Informed consent was obtained from all subjects, and ethical permission granted by the Stockholm County Regional Ethical Committee, with approval numbers 2005/1031-31/2, 2009/20-32, 2011/35-32 and 2012/132-32. Collaborative studies with the University of Colorado, Denver and University of Chicago were jointly approved by the respective local institutional review boards.

3.2 CLINICAL SAMPLING

3.2.1 Bronchoscopy with bronchoalveolar lavage

Bronchoscopy is a clinical procedure that enables visual inspection of the conducting airways and sampling of cell and tissue material from the lungs (Fig. 8). For sarcoidosis, histological assessment of cell composition and measurement

of CD4/CD8 ratio are routinely performed as part of the diagnostic procedure, for which reason patients are subjected to BAL as early as possible following admission to the clinic. Especially for LS patients, who experience a more acute but also self-limiting disease course, it is essential to perform bronchoscopy soon after disease onset, as the size and composition of the lymphocyte compartment change dramatically between active inflammation and remission, especially with regards to the percentage of TCR-specific $V\alpha2.3^{+}V\beta22^{+}$ T cells (182). For all studies, bronchoscopy and BAL were performed according to established procedures (223). Briefly, a flexible fibre-optic bronchoscope was introduced into the lower airways through the nose or mouth under local anaesthesia. Five aliquots of 50 ml sterile saline solution were instilled into the middle lobe of the right lung and recollected by aspiration. Cells in BALF were separated from recovered fluid by centrifugation, fixed on cytospin slides and stained with May-Grünwald Giemsa for calculation of leukocyte differential count. Cell-depleted BALF was further centrifuged to ensure removal of cell debris and stored at -80°C until further use, among others the study of solutes such as proteins, peptides, lipids and antibodies.

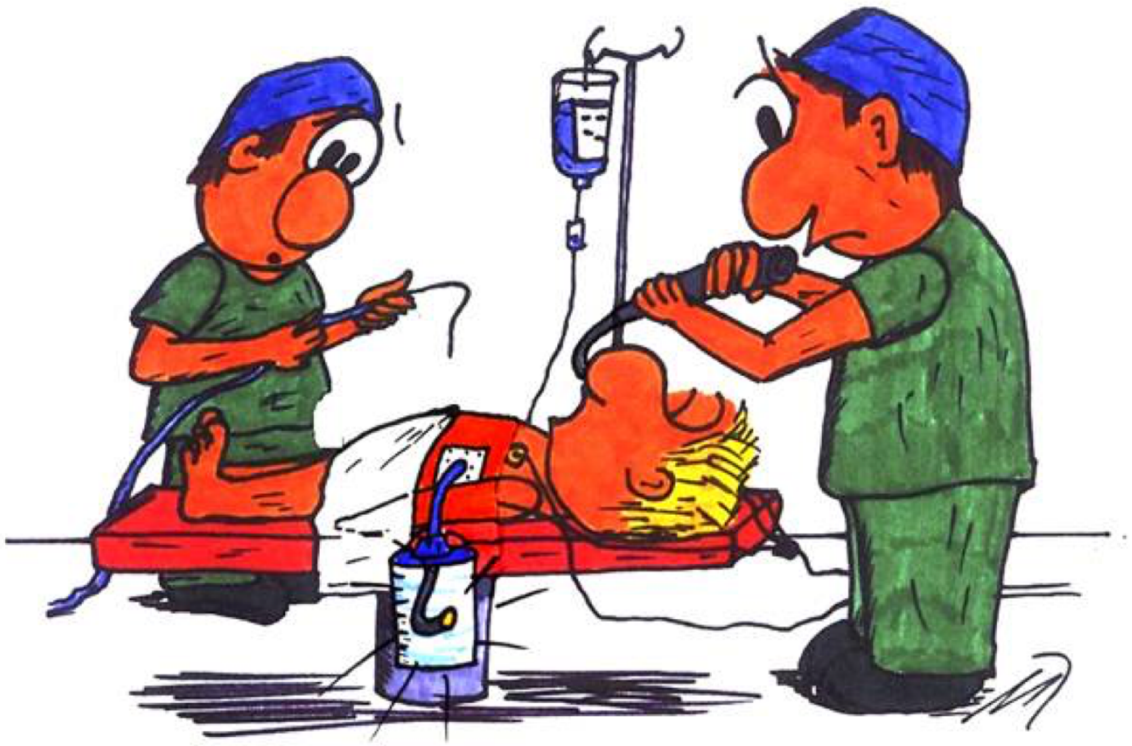

Figure 8 | Sampling of the lungs by bronchoscopy and bronchoalveolar lavage.

3.2.2 Measurements of lung function

In all patients, lung function was assessed by spirometry using standardised parameters (224) (Fig. 9-10). Dynamic measurements included vital capacity (VC), forced vital capacity (FVC) and forced expiratory volume during one second (FEV_1). In restrictive lung diseases such as sarcoidosis, a loss of pleural, parenchymal or extrapulmonary distensibility results in restricted capacity for expansion, whereby the total lung capacity (TLC) is reduced. This particularly prominent in patients with signs of pulmonary fibrosis, due to the gradual increase in scar tissue. In such instances, both FEV_1 and FVC are reduced, resulting in an approximately normal (≥80%), or even slightly elevated, FEV_1/FVC ratio, as the decline in FVC is usually more drastic than in FEV_1 (225) (Fig. 11).

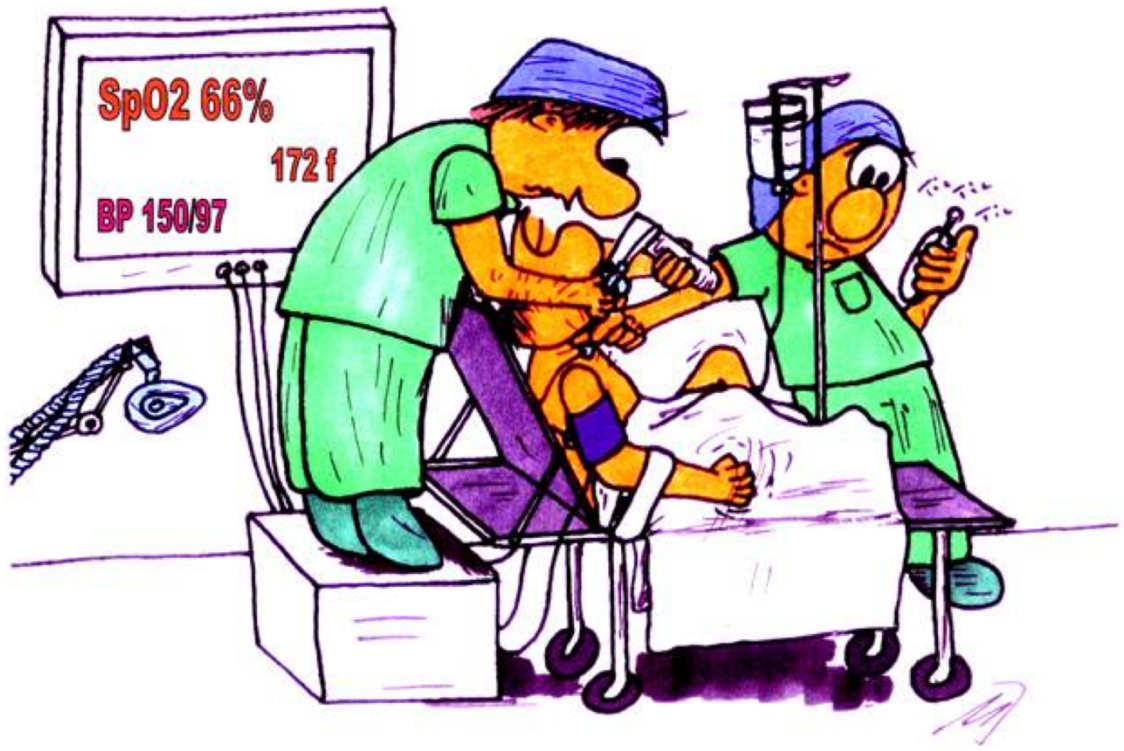

Figure 9 | Measurement of lung function by spirometry.

In addition to spirometry, the diffusing capacity of carbon monoxide (DLCO), which reflects the extent to which oxygen passes from the alveoli into the bloodstream, was measured. The test computes the difference in partial pressure between inspired and expired carbon monoxide; a small difference implies a low diffusing capacity, which may be caused by either restrictive or obstructive conditions. In the context of sarcoidosis, a significant decrease in DLCO may be indicative of more severe disease, e.g. with signs of pulmonary fibrosis (226).

As indicated by the table listings of patient data for the individual studies in this thesis, a reduction in DLCO is more frequently observed in non-LS patients, who also have a higher risk of developing chronic disease and permanent tissue remodelling.

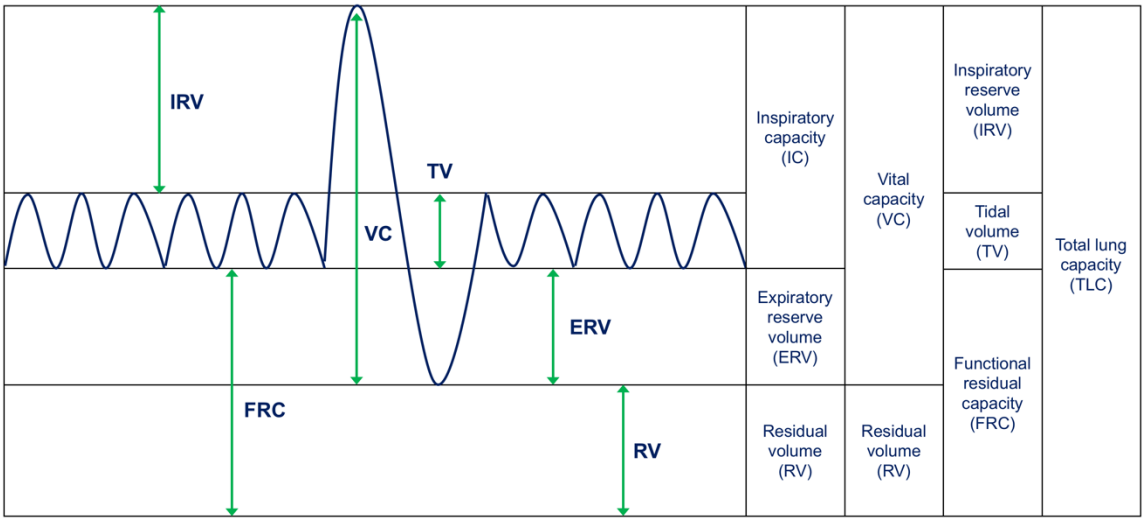

Figure 10 | Lung volumes measured during static spirometry. The measurement of the volume of air that can be in- and exhaled during a given time is an important indicator of lung function, and for identification of breathing patterns that may be indicative of pathogenic conditions. Total lung capacity (TLC) represents the volume in the lungs at maximum inflation. It is equivalent to the sum of vital capacity (VC), which is the volume of air exhaled after the deepest inhalation, and residual volume (RV), corresponding to the air remaining in the lungs after maximal exhalation.

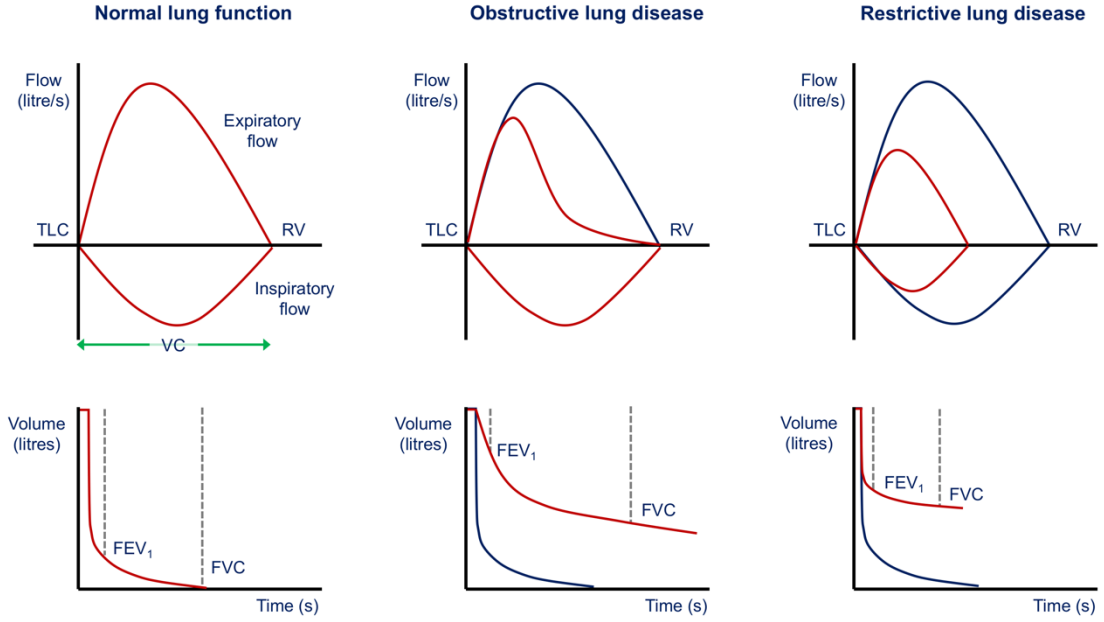

Figure 11 | Air flow and dynamic lung function parameters in the healthy lung, obstructive and restrictive disease. Red lines indicate the patterns of air flow and forced expiration in three different conditions, with blue lines offering a comparison to the situation in a healthy individual. TLC, total lung capacity; VC, vital capacity; RV, residual volume; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.

3.2.3 Whole blood, serum and peripheral blood mononuclear cells

Whole blood was collected from patients and healthy volunteers into sodium-heparinised tubes prior to bronchoscopy. Serum was isolated as part of the clinical routine investigation, immediately frozen and stored at -80°C prior to use for enzyme-linked immunosorbent assay (ELISA) (Paper V). Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque density gradient separation according to established protocols (184), and used as internal controls for patient-matched BALF cells in emulsion PCR (Paper IV). For Paper II, flow cytometry was run on whole blood following initial lysis of erythrocytes.

3.3 FLOW CYTOMETRY (PAPERS I-V)

Flow cytometry is a laser-based technique enabling simultaneous detection of various cellular components at the single-cell level, and identification of rare cell populations from a complex sample. In short, cells in solution are labelled with monoclonal antibodies coupled to fluorescent dyes and passed through a liquid stream under high pressure, allowing analysis of thousands of particles per second. Laser-mediated excitation of antibody-bound fluorochromes results in light emission at different (longer) wavelengths, which is captured by detectors, called photomultiplier tubes (PMT), placed around the stream. PMTs convert light into electrical signals that can then be digitalised and displayed as "fluorescence intensity". To avoid false positive detection of light "spill-over" from one emission channel to the next, a compensation setup is performed, where PMT voltage adjustment allows for maximum separation of positive and negative cell populations for each individual fluorochrome. In addition to the assessment of fluorescently labelled extra- and intracellular markers of choice, detectors parallel with the light beam measure forward scatter (FSC), which is proportional to cell size. In combination with side scatter (SSC), which is obtained by detectors placed perpendicularly to the stream and constitutes a measure of cell granularity, these two parameters offer the ability to discriminate major cell populations even in absence of additional labels. Furthermore, FSC and SSC can also be used to detect disproportionality between cell size and cell signal, aiding in discrimination of doublets, i.e. when more than one cell passes through the detector beam at the same time. The voltage pulse generated when a cell is excited by a laser is processed into three parameters: height (H), the maximum signal of a specific cell, which will change with adjustments in PMT voltage; width (W), the duration of the signal and a direct correlate of cell size, which is not affected by PMT voltage; and area (A), calculated from all H measurements made throughout the time (W) of cell

passage through the laser beam. Correlation of either FSC-A vs. FSC-H, FSC-H vs. FSC-W or SSC-H vs. SSC-W readily shows whether or not the electric signal of a cell is proportional to its size, positioning all singlet events on a diagonal display while doublets deviate from the 45° line, thus facilitating their exclusion from further analysis.

For all studies, the percentage of $CD4^+$ and $CD8^+$ $CD3^+$ T cells, respectively, as well as the percentage of $V\alpha2.3^+V\beta22^+$ $CD4^+$ $CD3^+$ T cells, in BALF of sarcoidosis patients and healthy individuals were measured by triple-laser, eight-colour flow cytometry using either a FACSCanto II, a FACSVerse, or a Fortessa X-20 (all supplied by Becton-Dickinson). As previously mentioned, a CD4/CD8 ratio >3.5 is considered a strong diagnostic indication for sarcoidosis. An expansion of TCR-specific cells was defined as a more than three-fold increase compared to the median frequency of the same TCR variable segment in peripheral blood of healthy individuals. For $V\alpha2.3^+$ T cells, this limit was set to >10% of all $CD4^+$ T cells in the lung (227); for $V\alpha2.3^+V\beta22^+$ $CD4^+$ T cells, to >0.5% (184). To adjust for spectral overlap between fluorochromes, compensation setup was performed using positive and negative compensation beads, as well as manual correction for varying degrees of BALF cell autofluorescence in individual samples. Singlets were identified through gating on FSC-A vs. FSC-H. Data analysis was performed using FlowJo X software.

In Paper I, the above-mentioned markers were complemented with CD69, a marker of T cell activation (228) and tissue-resident memory cells (187, 229), and CD27, a marker of T cell differentiation (186).

In Paper II, panels were designed to investigate $CD4^+$ T cell subsets and possibly multifunctionality. "Master" transcription factors T-bet (T_H1), RORγT (T_H17) and FoxP3 (T_{REG}) were complemented by chemokine receptors CXCR3 (T_H1), CCR6 (T_H17) and CCR4 (T_H2), as well as intracellular proliferation marker ki-67 and canonical cytokines IFNγ (T_H1) and IL-17A (T_H17). In this study, all markers were applied on cells from both BALF and whole blood derived from the same patient. A live/dead marker designed for intracellular staining was included in all panels for discrimination of viable cells.

For Papers III-V, CD4/CD8 ratio and TCR expression were evaluated as described above, prior to more comprehensive analysis by mass cytometry, emulsion PCR, and anti-vimentin antibody ELISA, respectively (detailed below).

3.4 TRIPLE-COLOUR FLUOROSPOT (PAPER II)

FluoroSpot is an extension of the ELISpot technology (230), facilitating measurement of cytokine-producing cells in solution following specific antigen and/or polyclonal stimulation. In summary, the method is based on the addition of cells and stimuli to a low-fluorescent polyvinylidene difluoride (PVDF) membrane pre-coated with cytokine-specific monoclonal antibodies. Upon stimulation, cells produce cytokines that diffuse into the membrane, forming spots. Each cytokine-producing cell is thereby detectable as a single spot following addition of secondary antibodies and visualisation in a specialised reading instrument, allowing for quantification of spot numbers in stimulated samples *versus* unstimulated controls. While ELISpot is restricted to the measurement of a single cytokine at a time, FluoroSpot utilises fluorescently labelled secondary antibodies, enabling simultaneous detection of up to three cytokines, as well as identification of cells producing more than one type of cytokine. In Paper II, FluoroSpot kits and monoclonal antibodies developed by Mabtech AB were used for concomitant detection of IFNγ, IL-2, IL-17A, IL-10 and IL-22 production by BALF cells stimulated with anti-CD3/anti-CD28, which specifically target T cells. Visualisation and analysis was performed using an AID SpectrumReader and ELISpot v.7.0 iSpot software supplied by Autoimmun Diagnostika GmbH. The basic assay principle is depicted in Fig. 12.

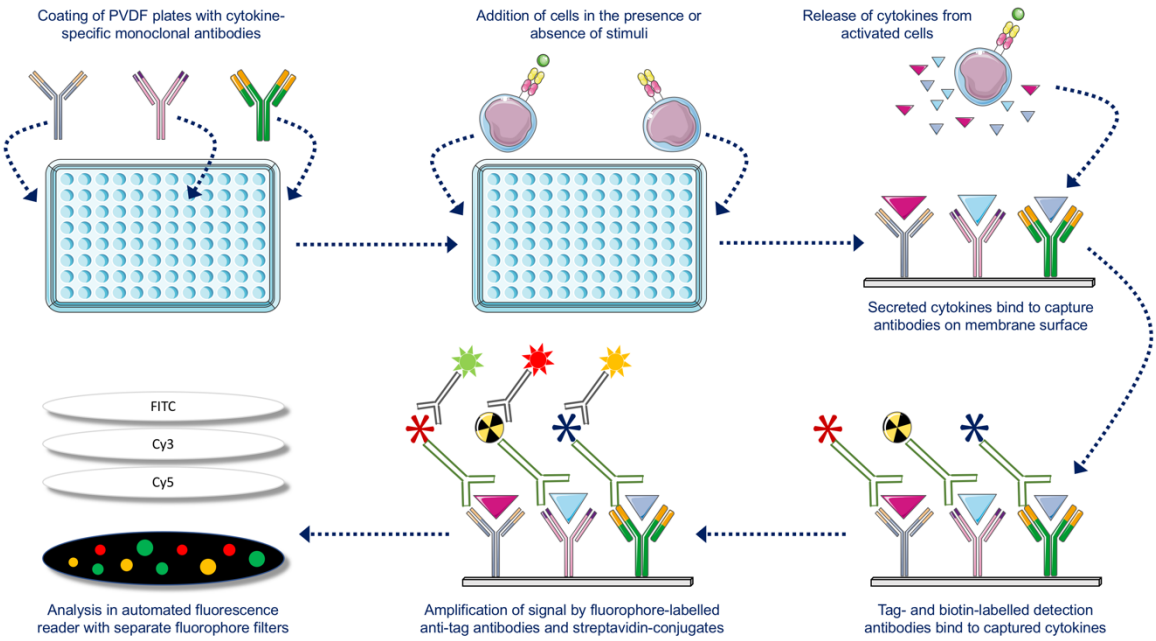

Figure 12 | Triple-colour FluoroSpot. Stepwise depiction of the FluoroSpot procedure, showing simultaneous coating of ethanol-treated PVDF membrane plates with three cytokine-specific monoclonal capture antibodies, followed by addition of cells and stimuli. After a suitable incubation time to allow for cytokine production, detection is performed in two sequential steps using secondary tag-labelled or biotinylated detection antibodies. Subsequent binding of fluorescently labelled anti-tag antibodies and streptavidin conjugates recognising biotin serves to amplify the detection signal. Final analysis is performed on dried plates utilising an automated fluorescence reader with separate filters for detection of the different fluorophores (FITC, Cy3 and Cy5, respectively).

3.5 MASS CYTOMETRY (PAPER III)

By labelling of antibodies with elemental isotopes rather than fluorochromes, mass cytometry circumvents the impediment of spectral overlap that confines flow cytometric analysis to a fixed number of cellular markers per laser, and effectively combines the technologies of mass spectrometry and flow cytometry. In brief, following incubation with cells, these antibody-bound metal conjugates are nebulised and run through an argon plasma, converting them to ions that can be detected by a time-of-flight mass spectrometer. This technique thereby offers a unique possibility for analysis of a considerably higher number of individual markers on the same cell, making its use ideal for unbiased explorative studies. In Paper III, mass cytometry was run on a CyTOF2 mass cytometer provided by Fluidigm, using a set panel of 33 markers supplemented with antibodies targeting the Vα2.3/Vβ22 TCR. In addition to T cell-specific markers, the panel included separation of singlets from doublets by measurement of DNA content in relation to cell length, and Cisplatin for assessment of cell viability. Consecutive steps of mass cytometry are visualised in Fig. 13.

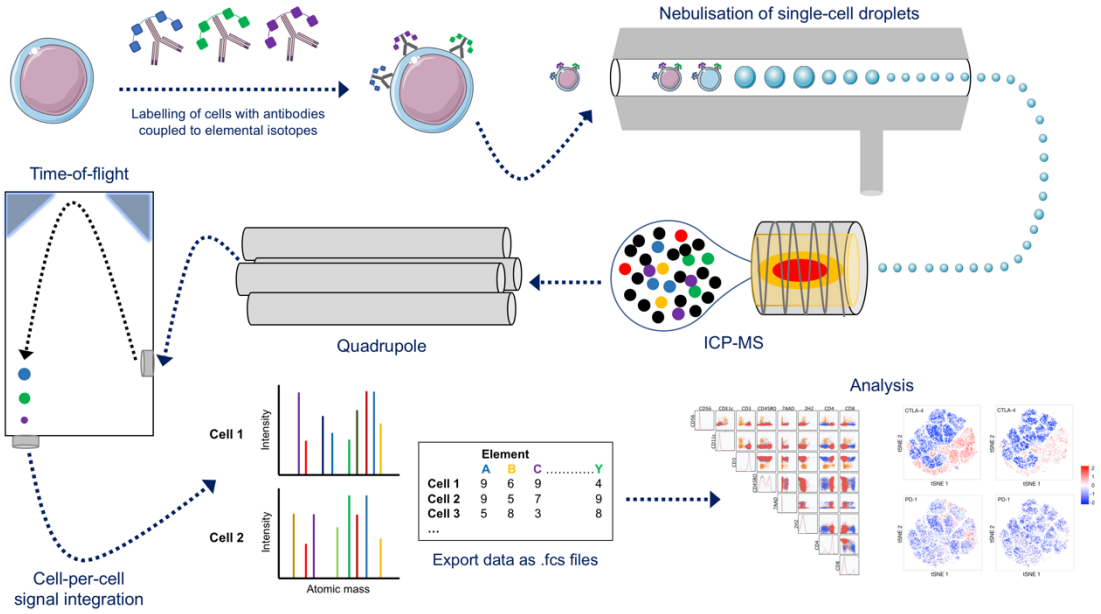

Figure 13 | Mass cytometry. Schematic flow-chart of the major steps involved in analysis of cells by mass cytometry, combining the technology of conventional flow cytometry with time-of-flight mass spectrometry. Cells are labelled with metal-conjugated antibodies, and samples in form of a liquid suspension are introduced into the instrument within a stream of argon gas, passing through a nebuliser to exit as aerosolised single-cell droplets. Resulting droplets are directed to the inductively coupled plasma-mass spectroscopy (ICP-MS) torch, undergoing successive vaporisation, atomisation and ionisation to generate an ion cloud, comprised of ions derived from both reporter metals and biological samples. Within the time-of-flight (TOF) mass analyser, ions are separated based on their mass-to-charge ratio and accelerated towards the detector. The quantity of each isotype, indirectly representing the expression of biological markers bound by metal-tagged antibodies, can thereby be determined for each individual cell. Data exported in the form of .fcs files are subsequently analysed using either routine flow cytometry analysis programmes, or more commonly, due to the high dimensionality and complexity of data, by multi-parameter analysis methods such as *t*-distributed stochastic neighbourhood embedding (*t*-SNE), principal component analysis (PCA) or spanning-tree progression analysis of density-normalised events (SPADE).

3.6 PCR, EPCR AND TCR SEQUENCING (PAPERS I AND IV)

In Paper I, mRNA was extracted from BALF cells from HLA-DRB1*03^{+} sarcoidosis patients, either following initial fluorescence-activated cell sorting (FACS) on Vα2.3^{+}Vβ22^{+} CD4^{+} T cells, or on bulk BALF. cDNA was generated and amplified by polymerase chain reaction (PCR) using primers for the Vα2.3 and Vβ22 TCR segments, respectively, in separate reactions. PCR products were then subjected to Next-Generation Sequencing (NGS), analysing the nucleotide sequences of the highly variable CDR3 segment, which is the region primarily involved in antigen binding. While a high degree of clonality of these cells could be established due to the remarkably low number of CDR3 sequences responsible for the vast majority of reads, as Vα2.3 and Vβ22 were assessed separately, the exact structure of the complete αβ TCR could not be determined. Therefore, an alternative approach was

applied in Paper IV, where $CD4^+$ T cells were sorted from HLA-DRB1*03^+ BALF and blood samples and whole TCR cDNA generated by emulsion PCR (ePCR) (231). Briefly, the first PCR step of a three-part reaction sees single cells immersed in individual oil emulsion droplets, with the addition of primers covering the entire range of TCR V segments and being designed for extension overlap. Through emulsion-mediated seclusion, cDNA from full αβ TCRs can be generated from single cells. After the emulsion is broken following termination of the first PCR reaction, blocking oligonucleotides are added for the second round of amplification, to ensure that only joint αβ sequences, and not unbound α's or β's, are further amplified. The final PCR introduces unique combinations of primers for each sample as barcodes for Illumina sequencing. The full TCR sequence is instrumental in the search for antigenic targets, and the identified CDR3 consensus sequences presented in Paper IV will serve as a foundation for generation of T cell hybridomas and subsequent screening against peptide positional scanning libraries (described below). A schematic overview of the ePCR procedure is shown in Fig. 14.

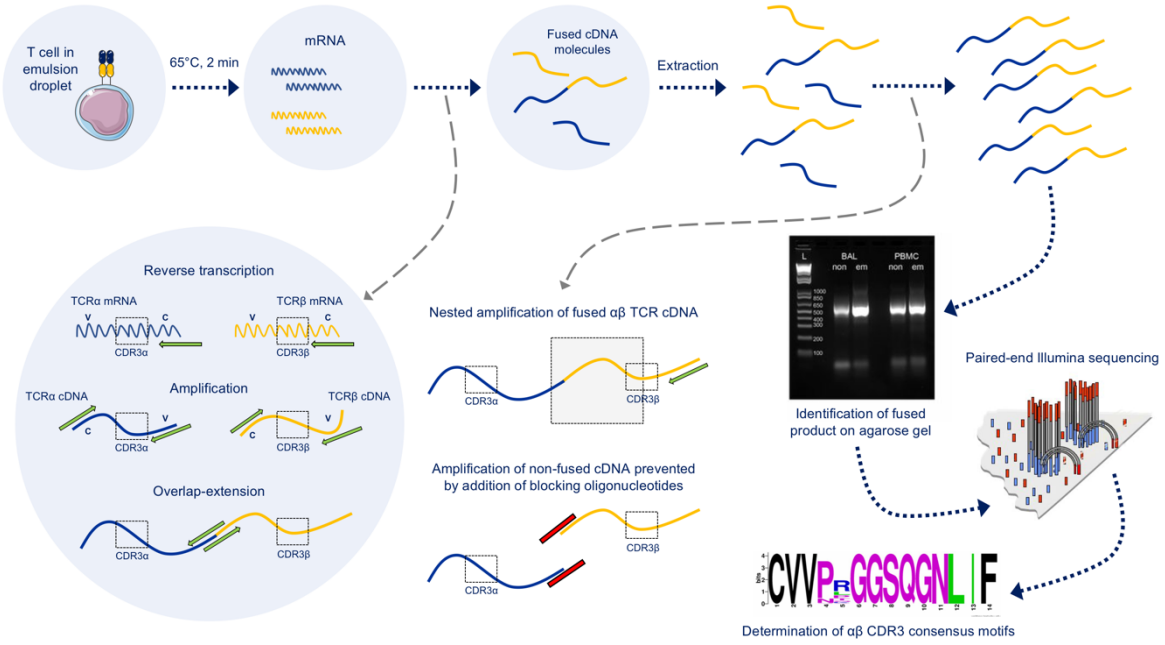

Figure 14 | **Emulsion PCR.** Stepwise depiction of the ePCR principle, showing immersion of single cells into emulsion droplets for subsequent extraction of TCR Vα and Vβ mRNA in a single-cell manner. Reverse transcription of mRNA into cDNA and subsequent amplification with extension overlap occurs within each droplet, ensuring that only TCR α and β chains expressed on the same cell are combined into cDNA molecules. Following extraction and DNA purification, nested amplification of fused TCR αβ cDNA is performed during a second round of PCR. The addition of blocking oligonucleotides ensures that free, non-fused α and/or β molecules are not amplified. A third round of PCR involves bar-coding for sequencing using unique primer combinations for each individual sample. The final PCR product is assessed on an agarose gel prior to submission for Illumina paired-end sequencing, the results of which are analysed to elucidate intra- and inter-patient recurrence of CDR3 sequences for the combined TCR α and β chains.

Importantly, while the NGS analysis in Paper I was selectively performed on the Vα2.3 and Vβ22 TCR segments, ePCR data in Paper IV was generated in an unbiased fashion, with all variable αβ TCR segments included in the sequencing process. Notably, the preferential association of Vα2.3 with Vβ22 and *vice versa* in HLA-DRB1*03^{+} patients could be confirmed by ePCR, enforcing the role of these cells in sarcoidosis immunopathology and presumably antigen recognition.

3.7 T CELL HYBRIDOMA GENERATION AND PEPTIDE LIBRARY SCREENING (PAPER IV AND BEYOND)

A T cell hybridoma is assembled by the insertion of a plasmid vector carrying the human TCR sequence of choice into an immortalised mouse cell line lacking innate TCR expression. This allows repeated stimulation assays to be performed using the same patient-derived TCR, but without the risk of depleting scarce clinical samples. Hybridomas are co-cultured with candidate peptides and APCs expressing HLA molecules of interest (in this case HLA-DRB1*03), preferably using Epstein-Barr virus (EBV)-transformed syngeneic PBMCs. Ten amino acid residue-long peptides, so-called decapeptides, are generated through sequential randomisation of residues where one position is always fixed, and other positions vary. Responsiveness of hybridomas to individual peptides is evaluated by means of an ELISA measuring IL-2 production. Combined data from screening of all peptide groups can then be used to deduce which amino acid is most likely to appear at which position, forming complete peptide sequences, here termed "mimotopes", which can be used as stimuli to test reactivity of primary patient cells. Ultimately, database comparison of mimotope sequences with those of microbial, environmental or self-derived proteins can facilitate discovery of biologically relevant antigenic candidates for further investigation. Fig. 15 outlines the experimental rationale for ePCR and ensuing experimental steps, including hybridoma generation and peptide library screening.

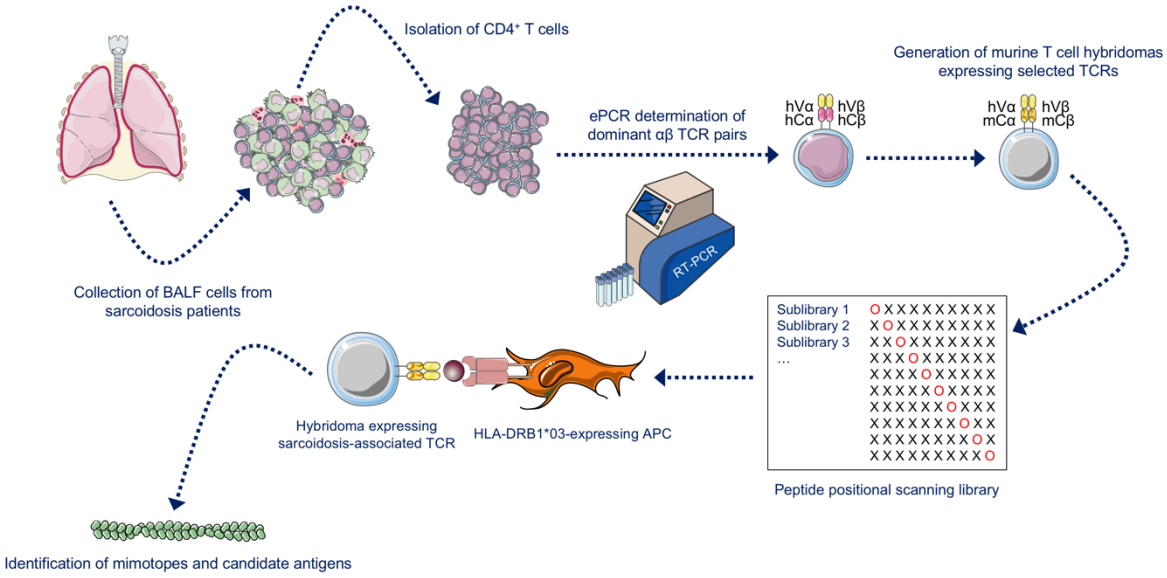

Figure 15 | Application of ePCR in T cell hybridoma generation and peptide library screening. The ePCR procedure constitutes a crucial first step in the ongoing process of determining peptide antigen specificity of $CD4^+$ T cells expressing the Vα2.3/Vβ22 TCR. Identified TCR Vα and Vβ CDR3 consensus sequences are introduced via a bacterial expression vector into immortalised murine T cell hybridomas lacking innate TCR expression. Hybridomas expressing human TCRs of interest are then co-cultured with APCs expressing HLA-DRB1*03 and decapeptides from a positional scanning library. Each of the ten sublibraries has one position fixed (O), while the others vary (X). Within each sublibrary, the fixed position can be any of the twenty known amino acids. Each sublibrary thus comprises 3.2×10^{11} different peptides, with a total of 6.4×10^{12} peptides in the complete library. Stepwise identification of preferred amino acid residues at each position generates "mimotope" sequences that can be used for identification of biologically available candidate antigens with similar properties, and subsequent stimulation tests on primary patient cells.

3.8 ANTI-VIMENTIN ANTIBODY ELISA (PAPER V)

Presence of e.g. proteins, peptides, antibodies or hormones in bodily fluids can be readily assessed by ELISA, where the antigen of interest is immobilised on a solid surface, commonly a polystyrene microtiter plate, either by direct adsorption to the surface or via capture by a specific antibody. Linkage of secondary antibodies to an enzyme enables detection of the target antigen by addition of an enzymatic substrate in the final step. Enzymatic activity generates a detectable signal, usually observed as a colour change in the plate and measured as optical density, which is relative to the quantity of antigen in the sample. In Paper V, the occurrence of IgG and IgA antibodies specific for the cytoskeletal protein vimentin, identified as a potential antigenic candidate in Paper I and preceding studies (116, 158), in BALF and serum from sarcoidosis patients and healthy controls was evaluated using an ELISA developed in-house at the University of Chicago (163). In all samples, antibody reactivity towards the full-length protein, as well as towards two amino (N)-terminal and one carboxyl (C)-terminal

truncations of vimentin, was measured. Subsequent correction for total IgG or IgA concentration yielded absolute concentrations of anti-vimentin antibodies (AVAs) in the same sample, which could be correlated to clinical or immunological parameters, such as HLA carriage, CD4/CD8 ratio, percentage of BALF $V\alpha2.3^+V\beta22^+$ $CD4^+$ T cells, or lung function.

3.9 IMMUNOFLUORESCENCE MULTI-COLOUR CONFOCAL MICROSCOPY (PAPER V)

Immunofluorescence microscopy is a powerful tool for visualisation of cells and extracellular components in tissue, and offers the ability to study *in situ* cell-cell co-localisation and interaction as a complement to analysis of cells and secreted components in solution. For Paper V, paraffin-embedded mucosal biopsies containing granulomatous tissue from sarcoid lungs, as well as corresponding samples from non-granulomatous healthy lungs, were fixed onto microscopy slides and stained for presence of $CD4^+$ T cells (using anti-CD3 and anti-CD4 antibodies), B cells (anti-CD20), vimentin and active proliferation (anti-ki-67). Tonsil tissue, known to harbour large organised clusters of B and T cells, was used as a positive staining control. Images were obtained using an 40X oil immersion objective on a Leica SP8 Laser Scanning microscope with 1024 x 1024 fields of view and colour-converted using Image J freeware.

3.10 STATISTICAL AND BIOINFORMATIC ANALYSIS

For all studies, univariate statistical analysis was performed in GraphPad Prism software using non-parametric tests, unless equal inter-group variances could be determined using an F-test. Depending on the particular assay, one- or two-way analysis of variance (ANOVA) tests were used for comparisons between groups. For individual comparisons, the two-tailed Mann-Whitney U test was applied, along with Wilcoxon's sign rank test for paired comparisons. Spearman's rank test was used for correlation analyses. In all instances, $p < 0.05$ was considered significant.

For Paper III, hierarchical cluster analysis of mass cytometry data was performed following manual gating on single, viable (DNA^+, $Cisplatin^-$) $CD3^+$ $CD4^+$ $CD8^-$ T cells. The Citrus algorithm (232) was used to identify $CD4^+$ T cell populations of different abundance in LS and non-LS samples based on the collective expression across all investigated markers. A relatively high false discovery rate (FDR) of <0.25 was chosen to allow for detection of differential regulation despite the small cohort size. Clusters were visualised by ACCENSE (233) and complemented with

dimensionality reduction by the *t*-distributed stochastic neighbourhood embedding (*t*-SNE) algorithm (234), run in R and RStudio (235), respectively.

3.11 TECHNICAL LIMITATIONS AND POTENTIAL STUDY BIAS

Despite their high sensitivity and specificity, all experimental methods harbour innate limitations that must be adequately addressed for correct analytical assessment. The studies presented in this thesis have all been conducted on human primary cells derived from BALF and blood, as well as cell-depleted BALF and serum. The use of human samples constitutes a challenge in itself, due to limited availability of material and the impracticality of introducing multiple points of sampling, especially for invasive procedures such as bronchoscopy. Data interpretation must therefore be approached with caution, as results only represent a snapshot of the current state of disease. Consequently, clinical characteristics of individual patients, such as disease onset, specific organ manifestations or chest radiographic stage, should also be taken into consideration during analysis to form as accurate and complete an image of the present immunological state relative to symptomatic presentation as possible. In sarcoidosis, routine HLA typing of patients is of particular importance due to the known influence of certain HLA alleles on disease presentation and outcome, and can provide essential information when combined with both molecular and clinical data. Conversely, HLA carriage can also introduce sampling bias, if disregarded during clinical assessment. Likewise, internationally, sarcoidosis patients are commonly treated as a single group contrasted to healthy individuals. In such case, the overarching discrepancy between the healthy and the inflamed lung may conceal true differences between patient subgroups that could be essential for prognostic evaluation or treatment indication, as well as for insights into molecular mechanisms of disease.

As previously mentioned, the main drawback of flow cytometry is its restriction to a fixed number of detectable fluorochromes per laser in the instrument, due to overlap of fluorochrome emission spectra, and thus an increasing need for compensation controls. The more markers included, the more difficult it therefore becomes to separate individual populations, especially when striving to analyse multiple markers present on the same cell. In the specific context of tissue-resident cells, it should also be noted that both flow cytometry and FluoroSpot analysis are further complicated by the intrinsic autofluorescence of alveolar macrophages, which constitute the dominant population in BALF. In contrast,

mass cytometry abolishes the need for extensive compensation measures, as the vaporisation of metal isotopes and subsequent detection of released ions do not cause any spectral overlap (Fig. 16). However, this relatively new technique is still limited by its demand for higher cell numbers (complicating its application on rare clinical samples), lower flow rate and thereby longer analysis time, more complex experimental procedure, and higher running costs, effectively making it unattainable for most average-sized laboratories. In comparison, flow cytometry offers higher sensitivity per individual marker, which is useful for focused analyses of already known study targets, while mass cytometry has the capacity to evaluate higher numbers of markers at the same time, enabling broader discovery studies of hitherto unknown molecular features. Notably, the sheer amount and complexity of data obtained from mass cytometric analyses set higher demands on computational processing. As life science becomes increasingly concerned with handling of large datasets, coming generations of scientists will likely be more diligently trained in bioinformatic approaches, in addition to laboratory methodology and biomedical theory. As yet, however, these skills are rarely found in combination, requiring collaborative efforts and potentially introducing difficulties in scientific communication, as well as interpretation of data from a clinically and biologically relevant perspective.

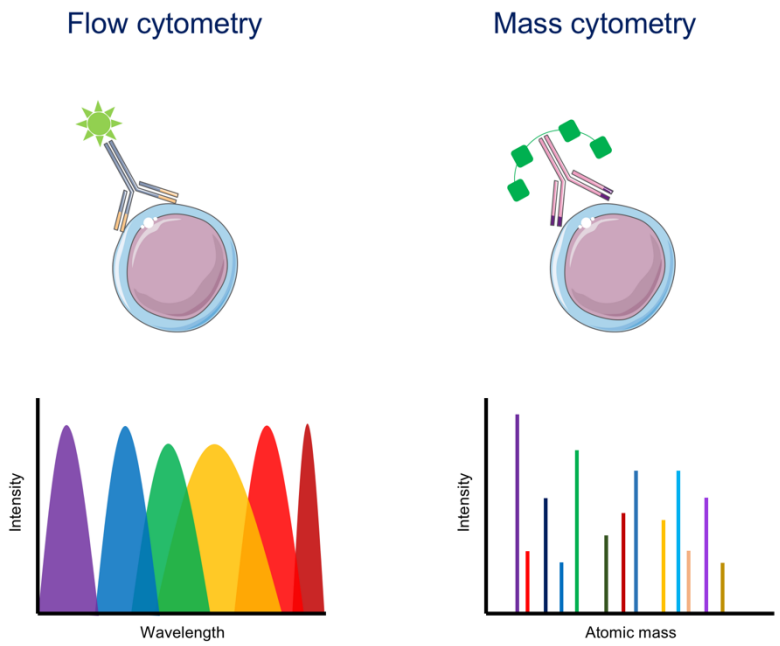

Figure 16 | Antibody-mediated detection in flow and mass cytometry. Simplified comparison of the underlying principle of antibody-mediated detection of cellular markers in flow and mass cytometry, respectively. While flow cytometry is limited by the need for fluorescence compensation due to spectral overlap between antibody-coupled fluorochromes, the mass cytometer allows for collection of mass spectral data in distinct channels separated by as little as one atomic mass unit. Through discrimination of isotopes with minimal overlap, mass cytometry vastly increases the number of markers that can be assessed simultaneously on a single cell.

Compared to flow cytometry, FluoroSpot offers detection of cytokine-producing cells with even higher sensitivity. Moreover, all cytokines produced throughout the course of incubation will be captured in the membrane and detected at the conclusion of the assay. This setup facilitates detection not only of early-response cytokines, but also of those where production is initiated later and therefore does not reach as high quantities within the same time frame. In contrast, cellular toxicity of protein-transporter blocking agent brefeldin A, which inhibits cytokine release from the cell and enables detection by intracellular staining, prevents cells subjected to analysis by flow cytometry from being kept in culture for extended periods of time (>18 h). This diminishes the time window for cytokine production, complicating analysis of late-response cytokines or cytokines produced in extremely low amounts. However, while flow cytometry allows for identification and further characterisation of the cells responsible for cytokine production by inclusion of additional markers in the analysis panel, FluoroSpot provides no means of further defining the cells, except for their responsiveness to e.g. T cell-specific stimuli. Direct quantitative comparisons between the different methods are therefore not possible, and they should rather be considered as complementary to one another. In Paper II, FluoroSpot was primarily used to demonstrate presence of a broader range of cytokines and in larger quantities than was possible to obtain by flow cytometry, while concomitant transcription factor and cytokine expression was deduced by the latter. In both cases, however, it is important to remember that different polyclonal stimuli will preferentially induce particular cytokine responses (236), which could potentially affect data interpretation.

Comparative studies of B and T cell responses, as highlighted by Paper V, are complicated by the inherent immunological differences between the two cell types. While T cell specificity is generally highly restricted to a few discrete HLA-peptide combinations, B cell responses are more multivalent, and multiple B cell clones producing antibodies with cross-reactive specificities are likely to expand in parallel in response to the same antigenic trigger. The presence of such cross-reactive antibodies is difficult to judge by means of an ELISA, and the correlation analyses made between humoral and cellular responses must therefore be considered as indicative of a joint B and T cell response, rather than directly quantifiable. In addition, tissue analysis by microscopy is, although highly informative, a largely non-quantitative approach, especially when applied to a limited set of samples, and mainly serves a visual purpose. Nevertheless, the observation of co-localised, proliferating B and T cells with extracellular vimentin

in the vicinity of sarcoid granulomas especially in HLA-DRB1*03^{+} patients, but less so in HLA-DRB1*03^{-} individuals and not in healthy lung tissue devoid of granulomatous structures, is highly suggestive of a coordinated antigen-driven response involving both branches of the adaptive immune system. Microscopy in this regard offers a valuable insight into the B cell compartment, which is not routinely considered due to the low numbers of B cells present in BALF, and complements the functional analysis of antibody production.

In summary, the use of several distinct, but reciprocal, methods is highly advisable, especially in translational science where human material is scarce and inter-patient variability is high. As evidenced by this thesis, the ability to confirm and expand on findings from one study in another using alternative techniques – exemplified by the identification of expanded Vα2.3^{+}Vβ22^{+} CD4^{+} T cells in BALF of HLA-DRB1*03^{+} patients by flow cytometry in Paper I and ePCR in Paper IV, the cytokine analyses performed with flow cytometry and FluoroSpot in Paper II, or the revelation of retained regulatory capacity of CD4^{+} T cells in LS compared to non-LS, supported by elevated IL-10 production in Paper II and expression of CTLA-4 and PD-1 in Paper III – strengthens the working hypotheses and highlights the benefit of performing validation experiments on the same cohort, as to extract as much information as possible from valuable clinical samples. For the research field as a whole, this also infers that although all methods are not available to all laboratories, studies can still be compared on a global level, as long as inclusion/exclusion criteria for patients are clearly accounted for.

4 RESULTS AND DISCUSSION

This section offers a short summary of the results of the individual studies, which are discussed separately and in more detail in the respective papers. Subsequent reflections on the collective findings of the entire thesis follow the red thread of antigen discovery and immunological differences between clinical phenotypes, and strive to provide a foundation for future research.

4.1 MAIN FINDINGS

4.1.1 Paper I

In the lungs of HLA-DRB1*03$^+$ patients with sarcoidosis, and most frequently LS, an accumulation of CD4$^+$ T cells expressing the Vα2.3/Vβ22 TCR was observed during active disease. These cells expressed high levels of CD69, which is an early marker of T cell activation following engagement of the TCR/CD3 complex in response to antigen (228). In addition, more recent studies have found CD69 to be one of the defining traits of tissue-resident memory cells (229). In this study, Vα2.3$^+$Vβ22$^+$ CD4$^+$ T cells expressed higher levels of CD69 than their CD4$^+$ T cell counterparts that did not express this TCR. Moreover, Vα2.3$^+$Vβ22$^+$ CD4$^+$ T cells showed markedly reduced expression of CD27, the expression of which is lost upon prolonged antigen exposure (186). TCR sequencing of the Vα2.3 and Vβ22 segments separately revealed only a few (<5) individual CDR3 sequences per patient, with identical or near-identical sequences being observed in different patients, and recurrence of certain charged amino acid residues at specific positions known to influence antigen binding. Together, these characteristics indicate a high degree of clonal expansion, in line with antigen-specific responses. Finally, a molecular 3D model of the TCR Vα2.3/Vβ22-HLA-DRB1*03 complex was generated based on the sequencing results. A peptide derived from the C-terminal end of the cytoskeletal protein vimentin was found to fit into the peptide-binding cleft, with connecting points towards all four HLA binding pockets, as well as the CDR3 loop of the Vβ22 chain, thus implicating vimentin as a potential autoantigen in sarcoidosis.

4.1.2 Paper II

CD4$^+$ T cells simultaneously expressing transcriptional regulators T-bet, associated with T_H1 responses, and T_H17 counterpart RORγT, were identified in the lungs, but not peripheral blood, of both sarcoidosis patients and healthy individuals. Frequencies of T-bet$^+$RORγT$^+$ cells were significantly higher in disease,

and especially in LS patients. In contrast, no differences were observed between the groups in terms of FoxP3 expression, and almost all FoxP3$^+$ cells were also positive for T-bet, indicating a selective regulation of T_H1-driven inflammation (36, 237), alternatively a transient upregulation of FoxP3, which has frequently been observed during effector T cell activation (34). In addition, concomitant expression of chemokine receptors CXCR3 and CCR6, but not CCR4, was elevated in the lungs compared to peripheral blood. T-bet$^+$RORγT$^+$ cells showed a markedly higher proliferative capacity, represented by expression of ki-67, which stains actively cycling cells, compared to T-bet$^-$RORγT$^-$ cells. Vα2.3$^+$Vβ22$^+$ CD4$^+$ T cells co-expressed T-bet and RORγT, as well as CXCR3 and CCR6, to a higher degree than CD4$^+$ T cells negative for this TCR. Moreover, T cells from LS patients produced a broader range of cytokines than non-LS T cells, with a higher percentage of cells producing IL-10, IL-17A, IL-2 and IL-22. In contrast, IFNγ production was significantly higher in non-LS T cells. T-bet$^+$RORγT$^+$ cells were able to produce IFNγ, IL-17A or both, and higher frequencies of these double-positive multifunctional cells correlated with non-chronic disease, suggesting a prominent role in tissue homeostasis and disease outcome.

4.1.3 Paper III

Building on to Paper II, a more comprehensive assessment of BALF CD4$^+$ T cells was achieved through mass cytometry and hierarchical cluster analysis. Nineteen CD4$^+$ T cell populations differing significantly in abundance between HLA-DRB1*03$^+$ LS and HLA-DRB1*03$^-$ non-LS patients were identified. Most prominently, LS clusters were defined by higher expression of regulatory receptors CTLA-4 and PD-1, as well as ICOS, while non-LS cells consistently showed higher expression of effector and activation markers HLA-DR, CD127 and CD39. Moreover, expression of adhesion molecule CD44 was consistently reduced in CD4$^+$ T cells from LS patients. Vα2.3$^+$ CD4$^+$ T cells demonstrated functional heterogeneity with expression of multiple inhibitory and/or co-stimulatory molecules, some of which appeared linked to co-expression of the Vβ22 TCR segment, or of other Vβ variants. Lastly, as shown by a near-complete overlap of CD8 and ki-67, CD8$^+$ T cells were discovered to proliferate to a markedly higher degree than CD4$^+$ T cells, suggesting active CTL involvement in disease progression.

4.1.4 Paper IV

As a follow-up to Paper I, ePCR was used for unbiased identification of joint TCR α and β chains from single $CD4^+$ T cells and the dominant αβ pairs associated with disease. The preferential pairing of Vα2.3 and Vβ22 in HLA-DRB1*03^+ LS patients observed by flow cytometry was confirmed by ePCR, as was the recurrence of specific CDR3 sequences within and between patients, but not in HLA-DRB1*03^- patients or controls. From this, both TCR α and β consensus sequences could be identified, constituting an important step forward in the search for candidate antigens that can be recognised by the Vα2.3/Vβ22 TCR, and for the first time providing a means of, within the scope of future studies, generating T cell hybridomas expressing entire patient-derived TCRs.

4.1.5 Paper V

Paper I hypothesised that a vimentin-derived peptide could act as a T cell autoantigen, which, together with the previously identified involvement of vimentin in B cell-mediated autoimmune diseases such as SLE and RA, prompted investigation of the B cell and anti-vimentin antibody repertoire also in sarcoidosis. This revealed higher IgG and IgA AVA titres in sarcoidosis patients compared to healthy controls, and markedly higher concentrations in BALF compared to blood. Moreover, AVA titres correlated with established diagnostic criteria such as CD4/CD8 ratio and percentage of $V\alpha2.3^+V\beta22^+$ $CD4^+$ T cells in BALF. Stratification of AVAs by target region, more specifically two N- and one C-terminal fractions, revealed a relatively higher degree of N-terminal-specific antibodies in healthy individuals, while HLA-DRB1*03^+ patients showed a shift towards C-terminal reactivity. Furthermore, HLA-DRB1*03^- patients presented with higher total Ig levels, but lower concentrations of specific AVAs, while the opposite was true for HLA-DRB1*03^+ patients. In the inflamed lung, extracellular vimentin was readily detected, and co-localised with T and B cells in the vicinity of granulomatous structures. Proliferation, as marked by ki-67 expression of both T and B cells, was again more pronounced in HLA-DRB1*03^+ patients. Together, this is indicative of a coordinated antigen-specific T and B cell response to vimentin in HLA-DRB1*03-mediated sarcoidosis.

4.2 DISCUSSION

4.2.1 The lung microenvironment promotes a T_H1/T_H17 phenotype that is augmented in sarcoidosis

While the strive to simplify nature as far as possible to better understand its complexity is a characteristic human trait, biological systems are built on dynamic processes and are therefore not readily classified into black-or-white scenarios. The emerging concept of T cell plasticity is the most recent example of how nature "fails" to conform to man-made definitions, and recent years have even revealed the existence of cells simultaneously expressing markers formerly believed to be unique for completely separate immunological compartments, e.g. "T cells" positive for both T cell co-receptor CD3 and B cell marker CD20 in MS (238). Indeed, the escalating number of more or less established subsets, distinguished only by subtle differences and repeatedly classified differently depending on the study conducted, may actually complicate rather than facilitate interpretation of the role of these cells in health and disease. Essentially, the notion of plasticity in itself means that subsets cannot be strictly defined over time, but only at the specific moment of sampling. In the context of human immunology, this is of particular importance as cells cannot be constantly monitored *in vivo* throughout their lifespan, or, consequently, throughout the course of disease.

Several studies conducted on peripheral blood have used combinations of chemokine receptors to define T cell subsets (194), but these definitions cannot readily be applied to tissue-resident cells. Chemokine receptors direct migration to the target organ where the cell will perform its designated function, and it is only reasonable that innate chemokine receptor expression of T cells will differ between tissue compartments even at steady-state. As evidenced by Paper II, a majority of $CD4^+$ T cells in both sarcoidosis patients and healthy individuals were found to express both T_H1-associated chemokine receptor CXCR3 and T_H17 equivalent CCR6 in BALF, but not in peripheral blood. This correlated well, but not perfectly, with expression of transcriptional regulators T-bet (T_H1) and RORγT (T_H17) in the lung. A similar discrepancy between the lung and circulation in terms of chemokine receptor expression has been shown by Ramstein and colleagues (193), where T_H subset definitions were based solely on chemokine receptor, and not transcription factor, expression. Notably, the presence of ~25% "T_H1/T_H17" hybrid cells also in healthy subjects suggests the situation in the sarcoid lung to be a mere extension of what is observed under homeostatic conditions. In Paper II, patients with non-chronic disease were found to present with higher frequencies

of T-bet$^+$RORγT$^+$ CD4$^+$ T cells compared to patients with chronic disease, further emphasising that these cells are not necessarily "pathogenic" in themselves. For instance, Lexberg *et al* previously argued that the combined effector repertoires of T_H1 and T_H17 cells might confer a physiological advantage at the single-cell level, and that such cells could be more efficient in orchestrating disease resolution (239). Importantly, however, a majority of the non-chronic patients in Paper II were diagnosed with LS, while the opposite was true for the chronic disease group. It still remains to be elucidated whether LS patients do have a better prognosis due to higher efficiency and differential functionality of T_H1/T_H17 cells, or whether LS and non-LS simply constitute two discrete, albeit similar, disease conditions that ought to be considered separately, with different factors contributing to disease presentation and outcome (some of which are discussed in the following sections), thus also influencing a discrepancy in T_H1/T_H17 polarisation and balance.

Nevertheless, in Crohn's disease, a granulomatous disorder affecting the gut, inhibition of IL-17A has been shown not to ameliorate, but rather to exacerbate inflammation (240), suggesting a protective effect of this cytokine and an overlap of T_H1, T_H17 and T_{REG} functionality in T cells residing in mucosal tissues. Instead, IFNγ may be the primary culprit in driving pathogenic processes (241). Secretion of IL-17A and IL-22 in the healthy gut (20) further highlights that more detailed investigation into immunological features of the healthy lung are strongly warranted. In addition, both these cytokines have been shown to have protective effects during *Mycobacterium tuberculosis*-induced granulomatous inflammation (242), and reduced IL-22 levels have been observed in patients with chronic sarcoidosis and IPF (21). Comprehensive investigation of functional parameters such as IL-23R expression on CD4$^+$ T cells would be worthwhile, as IL-23 appears to be a key facilitator of pro-inflammatory T_H17 responses (243). In its absence, RORγT$^+$ CD4$^+$ T cells can produce IL-10 and act to restrain inflammation (244). Genetic analysis have found profiles associated with the T_H17 and T_H22 differentiation pathways in LS, but not non-LS patients (95), and in Paper II, T cells from LS patients were observed to produce higher amounts of cytokines involved in immune regulation and mucosal homeostasis, most prominently IL-10, IL-22 and IL-17A. In contrast, non-LS T cells produced mainly IFNγ. Together, this strengthens the hypothesis that a combined T_H1/T_H17 response is central to pulmonary immunology, and that this response is enhanced in LS patients, but somehow skewed towards a more aggressive "T_H1-only" phenotype in non-LS patients. Accordingly, it is possible that the T_H1/T_H17-mediated pathogenicity

observed in e.g. RA, MS and psoriasis (13-17) is driven primarily by excessive IFNγ secretion, and that the pro- or anti-inflammatory functions of IL-17A are strongly dependent on the local tissue microenvironment.

4.2.2 Disease outcome depends on CD4⁺ T cell balance

Although of major interest for understanding immune homeostasis in the lung, the current studies show that the T_H1/T_H17 paradigm alone cannot fully explain the course of disease. Paper III outlines the pulmonary $CD4^+$ T cell compartment as highly dynamic, and reveals concomitant expression of several markers that may be of critical importance for disease outcome. Considering the entire $CD4^+$ T cell repertoire as a whole provides further incentive for reassessment of the role of T_{REG}s in sarcoidosis. Previous studies have reported elevated ICOS expression (200), as well as IL-10 production (201), in LS, suggestive of a more self-restrictive response, while in non-LS, a shift towards a purely effector-driven response seems to occur, exemplified e.g. by a reduction in T cell immunoglobulin mucin domain (TIM) molecules that have been proposed to negatively regulate T_H1 responses (245). In Paper II, the latter is represented by high IFNγ production and in Paper III by consistently upregulated expression of activation markers HLA-DR, CD127 and CD39.

Reduced expression of CTLA-4 on $CD4^+$ T cells in sarcoidosis compared to healthy individuals has been reported previously (145), and while only comprising one healthy control sample, Paper III confirmed this finding, as well as demonstrating a significant, further decrease in non-LS patients compared to LS. Retained CTLA-4 and PD-1 expression in HLA-DRB1*03^+ LS patients, who are known to have a particularly good prognosis, expands on the notion that the balance between immune activation and regulation is central to sarcoid inflammation. This hypothesis is further supported by the observed development of sarcoid-like granulomatous disease in patients receiving anti-CTLA-4 or anti-PD-1 therapy for malignancy (146-150), suggesting that abolishing this immune-dampening function propagates sarcoid inflammation. While some studies have argued for increased PD-1 expression to associate with reduced $CD4^+$ T cell proliferation and contribute to the pathogenicity of sarcoidosis in the context of an infection (246, 247), the view of sarcoidosis as an autoimmune disorder suggests that therapeutic PD-1 blockade would be counterproductive, if not directly harmful. In this scenario, as suggested by Paper III, among others, PD-1 is instead likely to be

upregulated as a means of controlling an adaptive immune response to persistent tissue (self-)antigens that the patient is unable to clear (206).

Moreover, these findings show that tissue-resident $CD4^+$ T cells may have the ability to perform certain regulatory functions without being classified as "true" T_{REG}s. In Paper II, this is exemplified by presence of proliferating T-bet^+FoxP3^+ $CD4^+$ T cells in BALF, a phenotype that may be involved in lung-specific immune regulation and protection against harmful (auto)immune reactions. This, again, stresses the importance of distinguishing cells characterised in the circulation from cells active in their respective target organs. Indeed, in all studies in this thesis, as well as in previous reports and a mass cytometry pilot analysis comparing paired BALF cells and PBMCs from sarcoidosis patients, BALF $CD4^+$ T cells emerge as markedly different, functionally and phenotypically, from their blood counterparts. In summary, these findings, and especially of Papers II and III in combination, highlight the importance of investigating all $CD4^+$ T cells rather than stratifying by subset-specific factors, as this restricts the search window and skews the information obtained, particularly in a tissue-specific context.

4.2.3 $CD4^+$ T cells are not the sole drivers of sarcoid inflammation

Not only are $CD4^+$ T cells responsible for propagating inflammation, but also, especially in LS, for mediating resolution, most prominently through secretion of inhibitory cytokines and expression of regulatory co-receptors. However, emerging evidence from the constituent papers of this thesis and others argue for more prominent involvement of other cellular and humoral responses than previously acknowledged. Remarkably, Paper III revealed a surprisingly active proliferation in the $CD8^+$ T cell compartment, while in comparison, $CD4^+$ T cells demonstrated much lower proliferative capacity. Furthermore, Paper III identified elevated CD57 expression in certain non-LS clusters, potentially reflecting T cells with a more advanced differentiation state, reduced replicative capacity but retained cytokine production, particularly of IFNγ (248-250), and cytotoxic functions (251). Given the recent observation that cytotoxic activity of $CD8^+$ T cells is augmented in non-LS patients (252), it is tempting to speculate that expression of regulatory receptors and secretion of inhibitory cytokines by $CD4^+$ T cells act to restrict cytotoxic $CD8^+$ T cell activity in LS lungs, while unrestrained CTL responses, including high IFNγ production, in non-LS effectively lead to chronic inflammation and persistent tissue damage. Indeed, it is possible that $CD8^+$ T cells constitute a more important contributor to progressive non-LS inflammation than

$CD4^+$ T cells, as suggested also by consistently lower CD4/CD8 ratios in non-LS patients.

In addition to $CD8^+$ T cells, B cells have received scarce attention throughout the history of sarcoidosis research, despite being observed to undergo somatic hypermutation in the lungs and to localise around sarcoid granulomas (253), as well as to directly correlate with the percentage of T cells in BALF (254). However, their exceptional capacity for antigen uptake and presentation make them exceedingly interesting in the context of antigen discovery, as well as in development of novel diagnostic and prognostic tools. Although not included in Paper III, mass cytometry analysis also indicated simultaneous expression of CXCR5, PD-1 and ICOS, among others, all of which are markers traditionally associated with T_{FH} cells. This suggests at least certain groups of $CD4^+$ T cells to engage in active interaction with B cells, and, in reference to the previous section, provides further rationale for their maintained PD-1 expression. In Paper V, B cell proliferation was shown to be particularly pronounced in HLA-DRB1*03^+ lungs, and to coincide with $CD4^+$ T cell activity. Moreover, while the total immunoglobulin content in BALF was higher in HLA-DRB1*03^- patients, the concentration of antibodies targeting specific vimentin epitopes was higher in HLA-DRB1*03^+ individuals, mirroring the image derived from T cell research, of a more antigen-driven, efficient and less aggressive immune response in these patients. The involvement of B cells and particularly class-switched, antigen-specific antibodies in sarcoid inflammation may be suggestive of memory development, although more detailed studies on B cell clonality, epitope specificity and surface markers are required to validate such statements. Intriguingly, in LS patients, disease recurrence years after complete clinical remission is only seen in HLA-DRB1*03^+ individuals (93), pointing to both a superior capacity for antigen clearance, but also possibly development of long-lived (self-)memory responses in these patients, such as has been demonstrated e.g. in experimental models of SLE (255, 256). Monitoring the AVA status of such patients during active disease, remission and potential relapse would therefore be most interesting in terms of prognostic assessment. Another notable observation, for continuity omitted from Paper V, was the significantly higher concentrations of AVAs in women compared to men. As female LS patients more frequently present with erythema nodosum (92), which is considered to be an immune complex-mediated condition (257), higher autoantibody titres in females might contribute to gender differences in symptomatic presentation. Together with the

gender-specific patterns of disease onset (71), this could provide further rationale for investigation of distinct pathogenic pathways in males and females.

4.2.4 Potential differences in granuloma formation and persistence between LS and non-LS

In Paper III, expression of adhesion molecule CD44 on $CD4^+$ T cells was found to differ significantly between HLA-DRB1*03^+ LS and HLA-DRB1*03^- non-LS patients, with T cells from LS patients expressing markedly lower levels compared to non-LS. CD44 exists in several isoforms due to alternative splicing and posttranslational modifications, and mediates adhesion to the endothelium and extracellular matrix. Its expression on lymphocytes in pulmonary sarcoidosis, as well as other mucosal disorders such as Crohn's disease, has been speculated to influence their homing and activation, in addition to stimulating macrophage differentiation (258). Furthermore, elevated levels of soluble CD44 have been demonstrated in conditions with an autoimmune background, including sarcoidosis (259, 260), suggesting involvement also in cell-cell communication. Oddly, few, if any, studies have been explicitly dedicated to identifying differences between sarcoidosis phenotypes with regards to the actual granuloma. As LS to a large extent is characterised by spontaneous resolution, any subtle variability in granuloma formation and maintenance could be of clinical relevance. Recently, Linke *et al* discovered increased activity of mechanistic target of rapamycin (mTOR) complexes in sarcoid granulomas of patients with active progressive disease, compared to patients undergoing remission (261). Intriguingly, mTOR complexes can be inhibited by CD44 blockade (262), suggesting a dual role of CD44 in adhesion as well as signal transduction during granuloma formation and persistence. Consistently reduced CD44 expression on LS $CD4^+$ T cells may thus contribute to a higher tendency of these granulomas to spontaneously dissolve. Importantly, in Paper V, the organisation of tertiary lymphoid structures was found to differ between HLA-DRB1*03^+ and HLA-DRB1*03^- patients, with clusters of co-localised, proliferating T and B cells being more pronounced in HLA-DRB1*03^+ patients. Together, these data suggest that the entire granuloma ought to be a focal point of future studies, with the aim of delving deeper specifically into the lymphocytic compartment. As stated in the previous sections, rather than directing all efforts at individual cells, coverage of multiple cell types and cell-cell interaction would provide more comprehensive understanding of the immunological situation in the tissue. In addition, further investigation of CD44-

mediated adhesion and/or signalling could prove valuable in identification of new prognostic markers, or even as a therapeutic target in progressive disease.

4.2.5 Vimentin is a potential autoantigenic target of T and B cell-mediated inflammation in the lung

In Paper I, the cytoskeletal protein vimentin was presented as a potential autoantigen, thanks to the ideal fit of a C-terminal peptide into the HLA-DRB1*03 peptide-binding cleft, its repeated isolation from HLA-DR molecules on alveolar macrophages from sarcoidosis patients (116, 158), and the potential of said peptide to stimulate IFNγ production by T cells (117). This was further expanded on in Paper V, where vimentin-specific antibodies were identified in BALF of sarcoidosis patients and particularly HLA-DRB1*03$^+$ individuals. The switch from vimentin N-terminal-specific antibodies in healthy individuals to C-terminal AVAs in HLA-DRB1*03$^+$ sarcoidosis patients is particularly intriguing with regards to the previously identified peptide. Molecular modelling in Paper I showed that this peptide is likely to be recognised by Vα2.3$^+$Vβ22$^+$ CD4$^+$ T cells when presented on HLA-DRB1*03, and in Paper V, the percentage of Vα2.3$^+$Vβ22$^+$ CD4$^+$ T cells in BALF correlated with specific AVA production. Overlapping or partially overlapping T and B cell epitopes have been identified e.g. in coeliac disease (263), indicative of ongoing T-B cell interaction, which in Paper V could be observed as marked T-B co-localisation and proliferation in the inflamed tissue. Efficient antigen processing and presentation by B cells may contribute to activation and differentiation of T cells in and surrounding the granuloma, while T cell help to B cells in turn drives somatic hypermutation and affinity maturation, ultimately resulting in development of memory cells.

In the context of sarcoidosis, it remains to be determined whether vimentin is a cause or effect of inflammation. Vimentin is known to be secreted from activated macrophages (264), which are abundant in the granulomatous environment, and Paper V verified that vimentin is readily available for uptake and processing in the lung. Its release could therefore be either a primary incident, following e.g. cell damage by intrusion of an external factor, or a secondary result of a triggering event driving granuloma formation and macrophage differentiation. In favour of vimentin being a primary antigen, Eberhardt and colleagues recently identified vimentin as a component of the Kveim reagent, and demonstrated a capacity of this vimentin to promote IFNγ production by T cells (157). Arguing against this are reports of anti-vimentin reactivity in other (auto)inflammatory conditions, such as

in lupus nephritis (163), RA (164) and IPF (265), suggesting that AVA reactivity is not disease-specific, but rather a sign of tissue autoimmunity, and possibly related to disease severity. This was exemplified by a weak, but detectable, association between reduced lung function and higher AVA titres in Paper V, similar to what was observed in IPF (265) and with kidney pathology in SLE (163). Alternatively, it is possible that anti-vimentin responses are a result of molecular mimicry following infection or exposure to environmental or occupational antigens. Further studies, such as are currently underway as a follow-up to Paper IV, are required to elucidate the true role of vimentin in sarcoidosis pathogenesis, but the results of this thesis at the very least suggest its involvement to be central to both T and B cell responses. $CD4^+$ T cell reactivity to vimentin was also demonstrated in IPF patients (265), and although this study did not stratify for HLA type or which region of the protein was targeted, it could imply an autoimmune component of IPF, and conceivably a general involvement of vimentin in pulmonary inflammation.

4.2.6 Functionally heterogeneous $V\alpha2.3^+V\beta22^+$ $CD4^+$ T cells are key mediators of disease outcome

The expansion of $V\alpha2.3^+V\beta22^+$ $CD4^+$ T cells specifically in HLA-DRB1*03^+ patients demonstrated in Papers I and IV implicates these cells as central to the process of antigen recognition. Papers I and II both delineated a high differentiation state of these cells, with high expression of CD69, T-bet, RORγT, CXCR3, CCR6, but not FoxP3 and only low levels of CD27. Previous studies on $V\alpha2.3^+$ T cells, prior to the discovery of joint expression with Vβ22, have clearly demonstrated that this expansion, which ranges up to ten-fold during active disease, unfailingly normalises after clinical recovery (182). This is consistent with an antigen-specific response that ebbs out following antigen clearance. Interestingly, a less dramatic, but significant, expansion of $V\alpha2.3^+$, but not $V\alpha2.3^+V\beta22^+$, $CD4^+$ T cells was observed in patients negative for HLA-DRB1*03, but positive for HLA-DRB3*01. These two HLA molecules are similar in structure and function, and able to present a highly similar peptide antigen repertoire (266), suggesting that Vα2.3 is able to pair with different Vβ segments to recognise different antigenic peptides from the same (or different) proteins, or, alternatively, to execute different functions. Accordingly, molecular modelling in Paper I visualised the connection between TCR and HLA-DRB1*03 as primarily mediated by the Vα2.3 segment, while Vβ22 mainly interacted with Vα2.3, but also with the HLA-bound peptide. Moreover, Paper III revealed differential expression of co-stimulatory and/or

-inhibitory receptors in $CD4^+$ T cells positive for either Vα2.3 alone or Vα2.3 and Vβ22, indicative of both effector and regulatory functions being performed in response to the same antigen. This is in line with the previous discussion of the immunological balance being maintained to a larger extent in HLA-DRB1*03^+ patients, and suggests these TCR-specific cells to influence both sides of the scale.

However, as carriage of *HLA-DRB1*03* is most frequent in LS patients, who have a favourable prognosis, and LS is speculated to be an autoimmune condition, the intriguing – and seemingly contradictory – question remains as to how autoreactive cells can contribute to disease resolution. One hypothesis is that with an intracellular antigen such as vimentin, antigen-specific cells might aid in its clearance from the extracellular environment, where protein components may drive unwanted inflammatory reactions. Differentiated $CD4^+$ T cells with the capacity to produce a broad range of cytokines and orchestrate a coordinated immune response could therefore cause an initially more intense reaction, represented by the acute symptomatic onset observed in LS, but also force resolution of granulomas and thereby disease. Along these lines, the T-B cell interaction demonstrated by Paper V would further suggest that AVAs may act to opsonise or neutralise extracellular vimentin, possibly participating in its removal. Consequently, in the absence of HLA-DRB1*03 and $Vα2.3^+Vβ22^+$ T cells, as in most non-LS patients, neither clearance nor immune regulation would be as efficient, resulting in prolonged IFNγ production, unrestricted cytotoxic $CD8^+$ T cell activity and eventually irreversible tissue damage that may progress to pulmonary fibrosis (Fig. 17). While it is conceivable that a range in antigen specificity exists among patients with different disease manifestations, e.g. in terms of organ involvement, it appears as though presentation on other HLA molecules is less successful in induction of effector cell differentiation, regardless of the antigenic source. Instead, as observed by a more pronounced lymphocytosis on BALF differential count and higher levels of overall antibody production, it propagates a potent, but less antigen-focused, lymphocytic response in HLA-DRB1*03^- patients. Nevertheless, despite the additional pieces of the sarcoidosis puzzle presented in this thesis, and the tools provided for further exploration of antigenic components that may drive inflammation, the true origin of disease remains a “blind spot” for future studies to shed light on.

5 CONCLUDING REMARKS

The collective works of this thesis highlight the complexity of sarcoidosis immunology and the heterogeneity of disease, ranging from subtle molecular differences to markedly diverse clinical symptoms to contrasting prognostic indications.

By using TCR-specific $V\alpha2.3^{+}V\beta22^{+}$ $CD4^{+}$ T cells as a vehicle for antigen discovery studies, the papers presented delineate a critical role for these cells in antigen recognition, but also plausibly clearance, resulting in an active, but transient, inflammatory response. The high differentiation state of these cells, exemplified by expression of multiple transcription factors, chemokine receptors, activation and regulatory markers, coupled with the remarkable degree of clonality observed within and between patients positive for the *HLA-DRB1*03* allele, are all strongly indicative of prolonged antigen exposure. Moreover, correlation of percentage of $V\alpha2.3^{+}V\beta22^{+}$ T cells with titres of specific antibodies suggest interaction between $CD4^{+}$ T cells and B cells in the inflamed lung, a concept rarely explored in sarcoidosis and with potential implications for improved prognostic assessment.

Clinically, LS and non-LS sarcoidosis are readily distinguished based on characteristic symptoms, but prediction of disease outcome is complicated by lack of ample knowledge of underlying immunological mechanisms. Furthermore, current therapeutic options are limited, unspecific and insufficient for managing the broad scope of symptoms. In this thesis, LS and non-LS are shown to harbour similar, but also significantly different immunological traits, which are likely to be instrumental in disease progression and outcome. The seemingly less aggressive, more antigen-driven, and more self-limiting immune response in LS relates well to the active inflammation and often spontaneous resolution observed in this patient group. Intriguingly, however, this disease form also exhibits features of autoimmunity, with selection for antigen-specific autoreactive T and B cells, as well as amplified levels of T_H1/T_H17 cells, frequently postulated to be central to pathogenesis in e.g. RA and MS. These findings emphasise immunological differences between organs, in turn suggesting that lessons learned from other diseases cannot be translated in their entirety to the sarcoid lung, and that further subclassification of patients is strongly warranted. Indeed, this would be of most benefit to non-LS patients, where prognosis is poorer and even less is known with regards to antigenic triggers. More comprehensive information on immunological

pathways will likely be essential in the ongoing effort to improve and personalise therapeutic strategies in sarcoidosis.

In summary, this thesis constitutes a small but significant step forward in the search for disease-initiating antigens and the understanding of molecular pathways underlying different clinical phenotypes of pulmonary sarcoidosis. Hopefully, the results and theories presented here may in time contribute to the illumination, and ultimately dispersing, of the "blind spot" that is sarcoidosis aetiology.

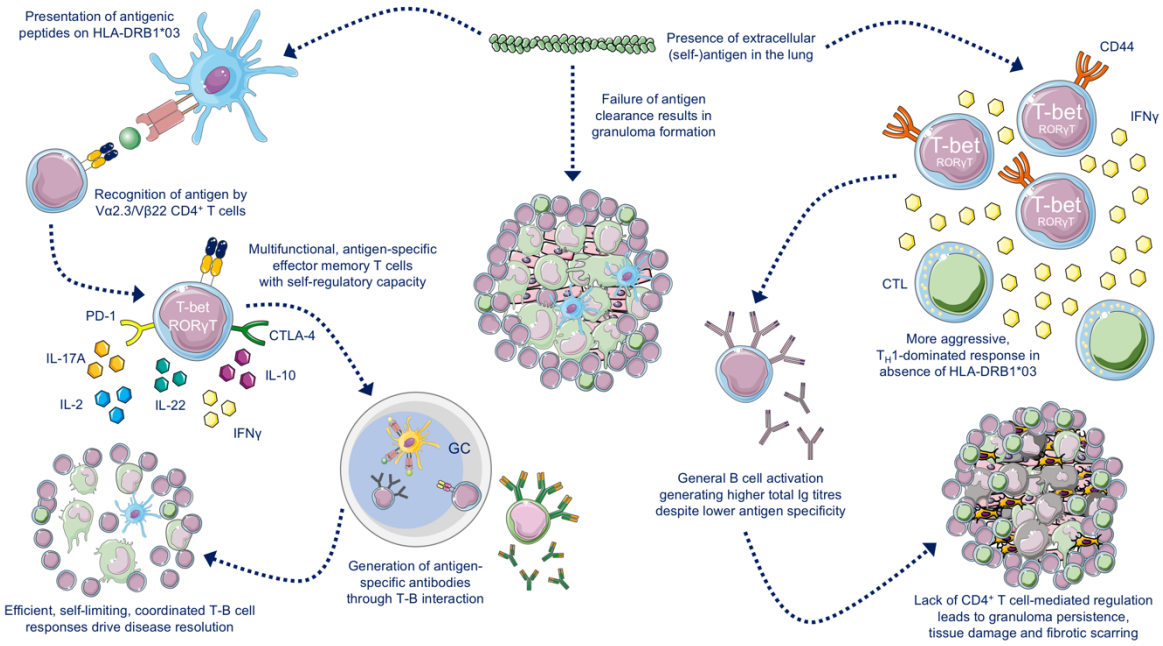

Figure 17 | Proposed mechanism of disease in LS and non-LS sarcoidosis. Graphic summary of the theorised immunological mechanisms in LS and non-LS, respectively, based on the findings presented in this thesis. The presence of a still unknown (self-)antigen in the pulmonary compartment triggers recognition and uptake by alveolar macrophages and DCs. In both disease forms, inability to completely clear the antigenic source results in its encapsulation and formation of characteristic granulomas. In LS, and particularly HLA-DRB1*03^{+} patients, TCR-specific CD4^{+} T cells are able to recognise peptide antigens and respond with a broad range of cytokine mediators. At the same time, these differentiated effector cells, expressing both T-bet and RORγT, appear able to restrict their own activity, as noted by maintained CD4^{+} T cell expression of regulatory receptors CTLA-4 and PD-1. CD4^{+} T cells in HLA-DRB1*03^{+} patients are further found to interact with B cells, giving rise to antigen-specific antibodies. Ultimately, an efficient antigen-driven T-B cell response contributes to spontaneous dissolution of granulomas and resolution of disease. In contrast, non-LS CD4^{+} T cells demonstrate a more aggressive, T_H1-dominant phenotype primarily expressing T-bet and producing high levels of IFNγ. Moreover, higher expression of adhesion marker CD44 may contribute to granuloma persistence, while lack of regulatory capacity in CD4^{+} T cells allows for unrestrained CTL proliferation, resulting in tissue damage. B cell activation occurs in absence of GC formation, resulting in higher total Ig concentration but a lower degree of antigen specificity. Together, these processes of unspecific yet potent lymphocytic activity produce a state of chronic inflammation and tissue disruption, with a risk of developing permanent fibrotic scarring.

6 FUTURE PERSPECTIVES

In the field of sarcoidosis, the primary aim of continued studies will be the search for, and identification of, disease-specific antigens. While current findings have described vimentin as a candidate target of both T cells and B cells in the lung, the involvement of vimentin in several other (auto)inflammatory conditions questions whether this is an occurrence specific for sarcoidosis or rather a tissue manifestation common to several chronic inflammatory conditions. Moreover, with regards to antigenic candidates, the inconclusive data presented over the past decades call for comprehensive, unbiased screening of possible peptide antigens recognised by the HLA-DRB1*03-TCR Vα2.3/Vβ22 complex, such as is now being performed in the next step of the study initiated in Paper I and continued in Paper IV. If successful, this approach could be further applied to other HLA-TCR combinations, e.g. in the context of HLA-DRB1*15 and chronic disease, or HLA-DRB1*04 and ocular sarcoidosis.

In addition, future studies should aim for more stringent and consistent subclassification of patients, not merely by LS and non-LS (which is, at present, rarely done outside Northern European countries), but also by HLA type, gender, presence of extrapulmonary symptoms and organ involvement. The disparity in symptomatic presentation between e.g. different ethnic groups, HLA backgrounds and genders indicate sarcoidosis to be not a single disease entity, but rather a spectrum of disorders, and proper classification of these conditions is essential for correct clinical management as well as for future research. Importantly, as indicated by the studies covered by this thesis, the traditional depiction of sarcoidosis as a solely $CD4^+$ T cell- and mainly T_H1-driven disease is no longer valid, and not only should regulatory features of T cells be further explored, but also the role of B cells, DCs and other tissue-resident immune cells with potential impact on antigen processing, presentation and downstream effector functions.

Ultimately, the long-term goal of both antigen discovery and detailed immunological studies should be to improve diagnostic and especially prognostic procedures, create more precise and individualised therapeutic options, and if possible, to develop preventive measures. For this reason, understanding not only the cause of disease, which may well differ between LS and non-LS, and within the non-LS group, but also the immunological response to this initial pathogenic event is critical. Importantly, data gathered from human samples offer a mere snapshot of a complex disease course, and must therefore be treated as such. As

exemplified by these and preceding studies, the cellular composition in the lung markedly differs from that of peripheral blood, wherewith sampling from the periphery is insufficient for proper assessment of the present immunological state of the patient. Despite the invasive nature of bronchoscopy, repeated investigations during follow-up and after clinical remission would be highly desirable from a research perspective, and provide information that could prove essential in unravelling the mechanisms that govern disease resolution and progression. Furthermore, systematic exploration into the affected tissue should be prioritised in order to gain insight into differences in granuloma formation and persistence between clinical phenotypes, which may be beneficial in the development of new treatments.

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They are done by a team of people.

- Steve Jobs

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8 REFERENCES

1. Abbas AK, Lichtman AH. Basic Immunology: Functions and Disorders of the Immune System. 2014.
2. Ahmed R, Bevan MJ, Reiner SL, Fearon DT. The precursors of memory: models and controversies. Nat Rev Immunol. 2009;9(9):662-8.
3. Chen K, Kolls JK. T cell-mediated host immune defenses in the lung. Annu Rev Immunol. 2013;31:605-33.
4. Rammensee HG. Chemistry of peptides associated with MHC class I and class II molecules. Curr Opin Immunol. 1995;7(1):85-96.
5. Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Dupont B, Erlich HA, et al. Nomenclature for factors of the HLA system, 2004. Tissue Antigens. 2005;65(4):301-69.
6. Robinson J, Waller MJ, Parham P, de Groot N, Bontrop R, Kennedy LJ, et al. IMGT/HLA and IMGT/MHC: sequence databases for the study of the major histocompatibility complex. Nucleic Acids Res. 2003;31(1):311-4.
7. Neefjes J, Jongsma ML, Paul P, Bakke O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. Nat Rev Immunol. 2011;11(12):823-36.
8. 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.
9. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. Pillars article: A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell. 2000. 100: 655-669. J Immunol. 2015;194(7):2961-75.
10. Murphy KM, Reiner SL. The lineage decisions of helper T cells. Nat Rev Immunol. 2002;2(12):933-44.
11. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? Nat Rev Immunol. 2010;10(4):225-35.
12. 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.
13. Zhang Y, Li Y, Lv TT, Yin ZJ, Wang XB. Elevated circulating Th17 and follicular helper CD4(+) T cells in patients with rheumatoid arthritis. APMIS. 2015;123(8):659-66.
14. Roeleveld DM, Koenders MI. The role of the Th17 cytokines IL-17 and IL-22 in Rheumatoid Arthritis pathogenesis and developments in cytokine immunotherapy. Cytokine. 2015;74(1):101-7.
15. Durham LE, Kirkham BW, Taams LS. Contribution of the IL-17 Pathway to Psoriasis and Psoriatic Arthritis. Curr Rheumatol Rep. 2015;17(8):55.
16. Malakouti M, Brown GE, Wang E, Koo J, Levin EC. The role of IL-17 in psoriasis. J Dermatolog Treat. 2015;26(1):41-4.

17. Luchtman DW, Ellwardt E, Larochelle C, Zipp F. IL-17 and related cytokines involved in the pathology and immunotherapy of multiple sclerosis: Current and future developments. Cytokine Growth Factor Rev. 2014;25(4):403-13.
18. Peters A, Lee Y, Kuchroo VK. The many faces of Th17 cells. Curr Opin Immunol. 2011;23(6):702-6.
19. Qu N, Xu M, Mizoguchi I, Furusawa J, Kaneko K, Watanabe K, et al. Pivotal roles of T-helper 17-related cytokines, IL-17, IL-22, and IL-23, in inflammatory diseases. Clin Dev Immunol. 2013;2013:968549.
20. O'Connor W, Jr., Zenewicz LA, Flavell RA. The dual nature of T(H)17 cells: shifting the focus to function. Nat Immunol. 2010;11(6):471-6.
21. Whittington HA, Armstrong L, Uppington KM, Millar AB. Interleukin-22: a potential immunomodulatory molecule in the lung. Am J Respir Cell Mol Biol. 2004;31(2):220-6.
22. Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, et al. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nat Med. 2008;14(3):275-81.
23. 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.
24. 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.
25. 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.
26. Koch SD, Uss E, van Lier RA, ten Berge IJ. Alloantigen-induced regulatory CD8+CD103+ T cells. Hum Immunol. 2008;69(11):737-44.
27. 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.
28. Ruprecht CR, Gattorno M, Ferlito F, Gregorio A, Martini A, Lanzavecchia A, et al. Coexpression of CD25 and CD27 identifies FoxP3+ regulatory T cells in inflamed synovia. J Exp Med. 2005;201(11):1793-803.
29. Bach JF. [Current concepts of autoimmunity]. Rev Neurol (Paris). 2002;158(10 Pt 1):881-6.
30. Brusko TM, Putnam AL, Bluestone JA. Human regulatory T cells: role in autoimmune disease and therapeutic opportunities. Immunol Rev. 2008;223:371-90.
31. Li P, Gao Y, Cao J, Wang W, Chen Y, Zhang G, et al. CD39+ regulatory T cells attenuate allergic airway inflammation. Clin Exp Allergy. 2015;45(6):1126-37.

32. Bending D, Pesenacker AM, Ursu S, Wu Q, Lom H, Thirugnanabalan B, et al. Hypomethylation at the regulatory T cell-specific demethylated region in CD25hi T cells is decoupled from FOXP3 expression at the inflamed site in childhood arthritis. J Immunol. 2014;193(6):2699-708.

33. Duhen T, Duhen R, Lanzavecchia A, Sallusto F, Campbell DJ. Functionally distinct subsets of human FOXP3+ Treg cells that phenotypically mirror effector Th cells. Blood. 2012;119(19):4430-40.

34. Wang J, Ioan-Facsinay A, van der Voort EI, Huizinga TW, Toes RE. Transient expression of FOXP3 in human activated nonregulatory CD4+ T cells. Eur J Immunol. 2007;37(1):129-38.

35. Yu F, Sharma S, Edwards J, Feigenbaum L, Zhu J. Dynamic expression of transcription factors T-bet and GATA-3 by regulatory T cells maintains immunotolerance. Nat Immunol. 2015;16(2):197-206.

36. Koch MA, Tucker-Heard G, Perdue NR, Killebrew JR, Urdahl KB, Campbell DJ. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. Nat Immunol. 2009;10(6):595-602.

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

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

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

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

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

42. Zhang F, Meng G, Strober W. Interactions among the transcription factors Runx1, RORgammat and Foxp3 regulate the differentiation of interleukin 17-producing T cells. Nat Immunol. 2008;9(11):1297-306.

43. Afzali B, Mitchell PJ, Edozie FC, Povoleri GA, Dowson SE, Demandt L, et al. CD161 expression characterizes a subpopulation of human regulatory T cells that produces IL-17 in a STAT3-dependent manner. Eur J Immunol. 2013;43(8):2043-54.

44. Beriou G, Costantino CM, Ashley CW, Yang L, Kuchroo VK, Baecher-Allan C, et al. IL-17-producing human peripheral regulatory T cells retain suppressive function. Blood. 2009;113(18):4240-9.

45. Chen K, Xu W, Wilson M, He B, Miller NW, Bengten E, et al. Immunoglobulin D enhances immune surveillance by activating antimicrobial, proinflammatory and B cell-stimulating programs in basophils. Nat Immunol. 2009;10(8):889-98.

46. Schwickert TA, Victora GD, Fooksman DR, Kamphorst AO, Mugnier MR, Gitlin AD, et al. A dynamic T cell-limited checkpoint regulates affinity-dependent B cell entry into the germinal center. J Exp Med. 2011;208(6):1243-52.

47. Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. SAP-controlled T-B cell interactions underlie germinal centre formation. Nature. 2008;455(7214):764-9.

48. Weisel FJ, Zuccarino-Catania GV, Chikina M, Shlomchik MJ. A Temporal Switch in the Germinal Center Determines Differential Output of Memory B and Plasma Cells. Immunity. 2016;44(1):116-30.

49. Taylor JJ, Pape KA, Jenkins MK. A germinal center-independent pathway generates unswitched memory B cells early in the primary response. J Exp Med. 2012;209(3):597-606.

50. Linterman MA, Beaton L, Yu D, Ramiscal RR, Srivastava M, Hogan JJ, et al. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. J Exp Med. 2010;207(2):353-63.

51. Nutt SL, Hodgkin PD, Tarlinton DM, Corcoran LM. The generation of antibody-secreting plasma cells. Nat Rev Immunol. 2015;15(3):160-71.

52. Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. Science. 2003;301(5638):1374-7.

53. Hillert J. Human leukocyte antigen studies in multiple sclerosis. Ann Neurol. 1994;36 Suppl:S15-7.

54. Kalman B, Lublin FD. The genetics of multiple sclerosis. A review. Biomed Pharmacother. 1999;53(8):358-70.

55. Gonzalez-Gay MA, Garcia-Porrua C, Hajeer AH. Influence of human leukocyte antigen-DRB1 on the susceptibility and severity of rheumatoid arthritis. Semin Arthritis Rheum. 2002;31(6):355-60.

56. Bodd M, Kim CY, Lundin KE, Sollid LM. T-cell response to gluten in patients with HLA-DQ2.2 reveals requirement of peptide-MHC stability in celiac disease. Gastroenterology. 2012;142(3):552-61.

57. Miller FW. Environmental agents and autoimmune diseases. Adv Exp Med Biol. 2011;711:61-81.

58. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016;16(10):626-38.

59. 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.

60. Hasenberg M, Stegemann-Koniszewski S, Gunzer M. Cellular immune reactions in the lung. Immunol Rev. 2013;251(1):189-214.

61. Hiemstra PS. Epithelial antimicrobial peptides and proteins: their role in host defence and inflammation. Paediatr Respir Rev. 2001;2(4):306-10.

62. Diamond G, Legarda D, Ryan LK. The innate immune response of the respiratory epithelium. Immunol Rev. 2000;173:27-38.

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

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

65. 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.

66. Herzenberg LA. B-1 cells: the lineage question revisited. Immunol Rev. 2000;175:9-22.

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

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

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

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

71. Arkema EV, Grunewald J, Kullberg S, Eklund A, Askling J. Sarcoidosis incidence and prevalence: a nationwide register-based assessment in Sweden. Eur Respir J. 2016;48(6):1690-9.

72. Mirsaeidi M, Machado RF, Schraufnagel D, Sweiss NJ, Baughman RP. Racial difference in sarcoidosis mortality in the United States. Chest. 2015;147(2):438-49.

73. 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.

74. 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.

75. Okamoto H, Mizuno K, Horio T. Monocyte-derived multinucleated giant cells and sarcoidosis. J Dermatol Sci. 2003;31(2):119-28.

76. Zissel G, Muller-Quernheim J. Cellular Players in the Immunopathogenesis of Sarcoidosis. Clin Chest Med. 2015;36(4):549-60.

77. Spector WG. Immunologic components of granuloma formation. Epithelioid cells, giant cells, and sarcoidosis. Ann N Y Acad Sci. 1976;278:3-6.

78. Dirican N, Anar C, Kaya S, Bircan HA, Colar HH, Cakir M. The clinical significance of hematologic parameters in patients with sarcoidosis. Clin Respir J. 2016;10(1):32-9.

79. Tutor-Ureta P, Citores MJ, Castejon R, Mellor-Pita S, Yebra-Bango M, Romero Y, et al. Prognostic value of neutrophils and NK cells in bronchoalveolar lavage of sarcoidosis. Cytometry B Clin Cytom. 2006;70(6):416-22.

80. Kjellin H, Silva E, Branca RM, Eklund A, Jakobsson PJ, Grunewald J, et al. Alterations in the membrane-associated proteome fraction of alveolar macrophages in sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 2016;33(1):17-28.

81. Ten Berge B, Kleinjan A, Muskens F, Hammad H, Hoogsteden HC, Hendriks RW, et al. Evidence for local dendritic cell activation in pulmonary sarcoidosis. Respir Res. 2012;13:33.

82. Schnerch J, Prasse A, Vlachakis D, Schuchardt KL, Pechkovsky DV, Goldmann T, et al. Functional TLR9 Expression and CXCR3 Ligand Release in Pulmonary Sarcoidosis. Am J Respir Cell Mol Biol. 2016.

83. 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.

84. 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.

85. 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.

86. Coury F, Annels N, Rivollier A, Olsson S, Santoro A, Speziani C, et al. Langerhans cell histiocytosis reveals a new IL-17A-dependent pathway of dendritic cell fusion. Nat Med. 2008;14(1):81-7.

87. Fais S, Burgio VL, Silvestri M, Capobianchi MR, Pacchiarotti A, Pallone F. Multinucleated giant cells generation induced by interferon-gamma. Changes in the expression and distribution of the intercellular adhesion molecule-1 during macrophages fusion and multinucleated giant cell formation. Lab Invest. 1994;71(5):737-44.

88. Costabel U, Hunninghake GW. ATS/ERS/WASOG statement on sarcoidosis. Sarcoidosis Statement Committee. American Thoracic Society. European Respiratory Society. World Association for Sarcoidosis and Other Granulomatous Disorders. Eur Respir J. 1999;14(4):735-7.

89. Zissel G. Cellular activation in the immune response of sarcoidosis. Semin Respir Crit Care Med. 2014;35(3):307-15.

90. Gilbert S, Steinbrech DS, Landas SK, Hunninghake GW. Amounts of angiotensin-converting enzyme mRNA reflect the burden of granulomas in granulomatous lung disease. Am Rev Respir Dis. 1993;148(2):483-6.

91. Lofgren S. Erythema nodosum: studies on etiology and pathogenesis in 185 adult cases. Acta Med Scand. 1946(124):1-197.

92. Grunewald J, Eklund A. Sex-specific manifestations of Lofgren's syndrome. Am J Respir Crit Care Med. 2007;175(1):40-4.

93. 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.

94. Grunewald J. HLA associations and Lofgren's syndrome. Expert Rev Clin Immunol. 2012;8(1):55-62.

95. Rivera NV, Ronninger M, Shchetynsky K, Franke A, Nöthen MM, Müller-Quernheim J, et al. High-density Genetic Mapping Identifies New Susceptibility Variants in Sarcoidosis Phenotypes and Shows Genomic-driven Phenotypic Differences. American Journal of Respiratory and Critical Care Medicine. 2015.

96. Rutherford RM, Brutsche MH, Kearns M, Bourke M, Stevens F, Gilmartin JJ. HLA-DR2 predicts susceptibility and disease chronicity in Irish sarcoidosis patients. Sarcoidosis Vasc Diffuse Lung Dis. 2004;21(3):191-8.

97. 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.

98. Grunewald J, Brynedal B, Darlington P, Nisell M, Cederlund K, Hillert J, et al. Different HLA-DRB1 allele distributions in distinct clinical subgroups of sarcoidosis patients. Respir Res. 2010;11:25.

99. Darlington P, Tallstedt L, Padyukov L, Kockum I, Cederlund K, Eklund A, et al. HLA-DRB1* alleles and symptoms associated with Heerfordt's syndrome in sarcoidosis. Eur Respir J. 2011;38(5):1151-7.

100. Spagnolo P, Sato H, Grutters JC, Renzoni EA, Marshall SE, Ruven HJ, et al. Analysis of BTNL2 genetic polymorphisms in British and Dutch patients with sarcoidosis. Tissue Antigens. 2007;70(3):219-27.

101. Swanson RM, Gavin MA, Escobar SS, Rottman JB, Lipsky BP, Dube S, et al. Butyrophilin-like 2 modulates B7 costimulation to induce Foxp3 expression and regulatory T cell development in mature T cells. J Immunol. 2013;190(5):2027-35.

102. Spagnolo P, Renzoni EA, Wells AU, Sato H, Grutters JC, Sestini P, et al. C-C chemokine receptor 2 and sarcoidosis: association with Lofgren's syndrome. Am J Respir Crit Care Med. 2003;168(10):1162-6.

103. Spagnolo P, Sato H, Grunewald J, Brynedal B, Hillert J, Mana J, et al. A common haplotype of the C-C chemokine receptor 2 gene and HLA-DRB1*0301 are independent genetic risk factors for Lofgren's syndrome. J Intern Med. 2008;264(5):433-41.

104. Fischer A, Valentonyte R, Nebel A, Nothnagel M, Muller-Quernheim J, Schurmann M, et al. Female-specific association of C-C chemokine receptor 5 gene polymorphisms with Lofgren's syndrome. J Mol Med (Berl). 2008;86(5):553-61.

105. Grunewald J, Idali F, Kockum I, Seddighzadeh M, Nisell M, Eklund A, et al. Major histocompatibility complex class II transactivator gene polymorphism: associations with Lofgren's syndrome. Tissue Antigens. 2010;76(2):96-101.
106. Karakaya B, van Moorsel CH, van der Helm-van Mil AH, Huizinga TW, Ruven HJ, van der Vis JJ, et al. Macrophage migration inhibitory factor (MIF) -173 polymorphism is associated with clinical erythema nodosum in Lofgren's syndrome. Cytokine. 2014;69(2):272-6.
107. Pabst S, Franken T, Schonau J, Stier S, Nickenig G, Meyer R, et al. Transforming growth factor-{beta} gene polymorphisms in different phenotypes of sarcoidosis. Eur Respir J. 2011;38(1):169-75.
108. Saidha S, Sotirchos ES, Eckstein C. Etiology of sarcoidosis: does infection play a role? Yale J Biol Med. 2012;85(1):133-41.
109. 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.
110. 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.
111. 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.
112. Ishige I, Eishi Y, Takemura T, Kobayashi I, Nakata K, Tanaka I, et al. Propionibacterium acnes is the most common bacterium commensal in peripheral lung tissue and mediastinal lymph nodes from subjects without sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 2005;22(1):33-42.
113. Negi M, Takemura T, Guzman J, Uchida K, Furukawa A, Suzuki Y, et al. Localization of propionibacterium acnes in granulomas supports a possible etiologic link between sarcoidosis and the bacterium. Mod Pathol. 2012;25(9):1284-97.
114. Newman KL, Newman LS. Occupational causes of sarcoidosis. Curr Opin Allergy Clin Immunol. 2012;12(2):145-50.
115. 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.
116. Wahlstrom J, Dengjel J, Persson B, Duyar H, Rammensee HG, Stevanovic S, et al. Identification of HLA-DR-bound peptides presented by human bronchoalveolar lavage cells in sarcoidosis. J Clin Invest. 2007;117(11):3576-82.
117. 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.

118. Padilla ML, Schilero GJ, Teirstein AS. Donor-acquired sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 2002;19(1):18-24.
119. Chen ES, Moller DR. Etiologies of Sarcoidosis. Clin Rev Allergy Immunol. 2015;49(1):6-18.
120. Dubaniewicz A, Kalinowski L, Dudziak M, Kalinowska A, Singh M. Peroxynitrite in Sarcoidosis: Relation to Mycobacterium Stationary Phase. Adv Exp Med Biol. 2015;866:41-9.
121. Moller DR. Potential etiologic agents in sarcoidosis. Proc Am Thorac Soc. 2007;4(5):465-8.
122. Saboor SA, Johnson NM, McFadden J. Detection of mycobacterial DNA in sarcoidosis and tuberculosis with polymerase chain reaction. Lancet. 1992;339(8800):1012-5.
123. Demirkok SS, Basaranoglu M, Coker E, Karayel T. Seasonality of the onset of symptoms, tuberculin test anergy and Kveim positive reaction in a large cohort of patients with sarcoidosis. Respirology. 2007;12(4):591-3.
124. 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.
125. Bresnitz EA, Strom BL. Epidemiology of sarcoidosis. Epidemiol Rev. 1983;5:124-56.
126. Barnard J, Rose C, Newman L, Canner M, Martyny J, McCammon C, et al. Job and industry classifications associated with sarcoidosis in A Case-Control Etiologic Study of Sarcoidosis (ACCESS). J Occup Environ Med. 2005;47(3):226-34.
127. Jordan HT, Stellman SD, Prezant D, Teirstein A, Osahan SS, Cone JE. Sarcoidosis diagnosed after September 11, 2001, among adults exposed to the World Trade Center disaster. J Occup Environ Med. 2011;53(9):966-74.
128. Liu H, Patel D, Welch A, Wilson C, Mroz M, Li L, et al. Association between Occupational Exposures and Sarcoidosis: an Analysis from Death Certificates in the United States, 1988-1999. Chest. 2016.
129. 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.
130. Guidotti TL, Prezant D, de la Hoz RE, Miller A. The evolving spectrum of pulmonary disease in responders to the World Trade Center tragedy. Am J Ind Med. 2011;54(9):649-60.
131. Ye RD, Sun L. Emerging functions of serum amyloid A in inflammation. J Leukoc Biol. 2015;98(6):923-9.
132. Ivanisevic J, Kotur-Stevuljevic J, Stefanovic A, Spasic S, Vucinic Mihailovic V, Videnovic Ivanov J, et al. Association of serum amyloid A and oxidative stress

with paraoxonase 1 in sarcoidosis patients. Eur J Clin Invest. 2016;46(5):418-24.

133. Bargagli E, Magi B, Olivieri C, Bianchi N, Landi C, Rottoli P. Analysis of serum amyloid A in sarcoidosis patients. Respir Med. 2011;105(5):775-80.

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

135. Rybicki BA, Iannuzzi MC, Frederick MM, Thompson BW, Rossman MD, Bresnitz EA, et al. Familial aggregation of sarcoidosis. A case-control etiologic study of sarcoidosis (ACCESS). Am J Respir Crit Care Med. 2001;164(11):2085-91.

136. Sverrild A, Backer V, Kyvik KO, Kaprio J, Milman N, Svendsen CB, et al. Heredity in sarcoidosis: a registry-based twin study. Thorax. 2008;63(10):894-6.

137. Johard U, Eklund A. Recurrent Lofgren's syndrome in three patients with sarcoidosis. Sarcoidosis. 1993;10(2):125-7.

138. Mana J, Montero A, Vidal M, Marcoval J, Pujol R. Recurrent sarcoidosis: a study of 17 patients with 24 episodes of recurrence. Sarcoidosis Vasc Diffuse Lung Dis. 2003;20(3):212-21.

139. Foulon G, Wislez M, Naccache JM, Blanc FX, Rabbat A, Israel-Biet D, et al. Sarcoidosis in HIV-infected patients in the era of highly active antiretroviral therapy. Clin Infect Dis. 2004;38(3):418-25.

140. Morris DG, Jasmer RM, Huang L, Gotway MB, Nishimura S, King TE, Jr. Sarcoidosis following HIV infection: evidence for CD4+ lymphocyte dependence. Chest. 2003;124(3):929-35.

141. Viana de Andrade AC, Brito EA, Harris OM, Viana de Andrade AP, Leite MF, Pithon MM. Development of systemic sarcoidosis and xanthoma planum during multiple sclerosis treatment with interferon-beta 1a: Case Report. Int J Dermatol. 2015;54(5):e140-5.

142. Chiang CH, Lai FJ. Sarcoidosis on the injection sites following treatment of interferon-alpha and ribavirin for hepatitis C. J Formos Med Assoc. 2014;113(12):981-2.

143. Jeon EK, Hong J, Hong SH, Kang JY, Park G, Ahn MI, et al. First reported case of interferon-alpha-induced sarcoidosis in an Asian patient with malignant melanoma. Asia Pac J Clin Oncol. 2014.

144. Walker LS, Sansom DM. The emerging role of CTLA4 as a cell-extrinsic regulator of T cell responses. Nat Rev Immunol. 2011;11(12):852-63.

145. Broos CE, van Nimwegen M, In 't Veen JC, Hoogsteden HC, Hendriks RW, van den Blink B, et al. Decreased Cytotoxic T-Lymphocyte Antigen 4 Expression on Regulatory T Cells and Th17 Cells in Sarcoidosis: Double Trouble? Am J Respir Crit Care Med. 2015;192(6):763-5.

146. Birnbaum MR, Ma MW, Fleisig S, Packer S, Amin BD, Jacobson M, et al. Nivolumab-related cutaneous sarcoidosis in a patient with lung adenocarcinoma. JAAD Case Rep. 2017;3(3):208-11.

147. Danlos FX, Pages C, Baroudjian B, Vercellino L, Battistella M, Mimoun M, et al. Nivolumab-Induced Sarcoid-Like Granulomatous Reaction in a Patient With Advanced Melanoma. Chest. 2016;149(5):e133-6.

148. Montaudie H, Pradelli J, Passeron T, Lacour JP, Leroy S. Pulmonary sarcoid-like granulomatosis induced by nivolumab. Br J Dermatol. 2017;176(4):1060-3.

149. Suozzi KC, Stahl M, Ko CJ, Chiang A, Gettinger SN, Siegel MD, et al. Immune-related sarcoidosis observed in combination ipilimumab and nivolumab therapy. JAAD Case Rep. 2016;2(3):264-8.

150. Vogel WV, Guislain A, Kvistborg P, Schumacher TN, Haanen JB, Blank CU. Ipilimumab-induced sarcoidosis in a patient with metastatic melanoma undergoing complete remission. J Clin Oncol. 2012;30(2):e7-e10.

151. Kveim A. En ny og spesifikk kutan-reaksjon ved Boecks sarcoid [A new and specific cutaneous reaction in Boeck's sarcoid]. Nord Med. 1941;9(169).

152. Kveim A. Some remarks on the aetiology of Boeck's sarcoid. Acta Derm Venereol. 1948;28(2):169.

153. Chase MW. The preparation and standardization of Kveim testing antigen. Am Rev Respir Dis. 1961;84(5)Pt 2:86-8.

154. Richter E, Kataria YP, Zissel G, Homolka J, Schlaak M, Muller-Quernheim J. Analysis of the Kveim-Siltzbach test reagent for bacterial DNA. Am J Respir Crit Care Med. 1999;159(6):1981-4.

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

156. Lindahl M, Andersson O, Ripe E, Holm G. Stimulation of bronchoalveolar lavage (BAL) and blood lymphocytes by Kveim antigen, tuberculin and concanavalin A in sarcoidosis. Br J Dis Chest. 1988;82(4):386-93.

157. Eberhardt C, Thillai M, Parker R, Siddiqui N, Potiphar L, Goldin R, et al. Proteomic Analysis of Kveim Reagent Identifies Targets of Cellular Immunity in Sarcoidosis. PLoS One. 2017;12(1):e0170285.

158. Heyder T, Kohler M, Tarasova NK, Haag S, Rutishauser D, Rivera NV, et al. Approach for Identifying Human Leukocyte Antigen (HLA)-DR Bound Peptides from Scarce Clinical Samples. Mol Cell Proteomics. 2016;15(9):3017-29.

159. Morris DL, Fernando MM, Taylor KE, Chung SA, Nititham J, Alarcon-Riquelme ME, et al. MHC associations with clinical and autoantibody manifestations in European SLE. Genes Immun. 2014;15(4):210-7.

160. Bernstrand C, Carstensen H, Jakobsen B, Svejgaard A, Henter JI, Olerup O. Immunogenetic heterogeneity in single-system and multisystem langerhans cell histiocytosis. Pediatr Res. 2003;54(1):30-6.

161. Mastaglia FL, Needham M, Scott A, James I, Zilko P, Day T, et al. Sporadic inclusion body myositis: HLA-DRB1 allele interactions influence disease risk and clinical phenotype. Neuromuscul Disord. 2009;19(11):763-5.

162. Rothwell S, Cooper RG, Lundberg IE, Miller FW, Gregersen PK, Bowes J, et al. Dense genotyping of immune-related loci in idiopathic inflammatory myopathies confirms HLA alleles as the strongest genetic risk factor and suggests different genetic background for major clinical subgroups. Ann Rheum Dis. 2015.

163. Kinloch AJ, Chang A, Ko K, Henry Dunand CJ, Henderson S, Maienschein-Cline M, et al. Vimentin is a dominant target of in situ humoral immunity in human lupus tubulointerstitial nephritis. Arthritis Rheumatol. 2014;66(12):3359-70.

164. Wegner N, Lundberg K, Kinloch A, Fisher B, Malmstrom V, Feldmann M, et al. Autoimmunity to specific citrullinated proteins gives the first clues to the etiology of rheumatoid arthritis. Immunol Rev. 2010;233(1):34-54.

165. Vossenaar ER, Despres N, Lapointe E, van der Heijden A, Lora M, Senshu T, et al. Rheumatoid arthritis specific anti-Sa antibodies target citrullinated vimentin. Arthritis Res Ther. 2004;6(2):R142-50.

166. Kinloch AJ, Lundberg KE, Moyes D, Venables PJ. Pathogenic role of antibodies to citrullinated proteins in rheumatoid arthritis. Expert Rev Clin Immunol. 2006;2(3):365-75.

167. Cervera R, Asherson RA. Antiphospholipid syndrome associated with infections: clinical and microbiological characteristics. Immunobiology. 2005;210(10):735-41.

168. Gudjonsson JE, Johnston A, Sigmundsdottir H, Valdimarsson H. Immunopathogenic mechanisms in psoriasis. Clin Exp Immunol. 2004;135(1):1-8.

169. Cusick MF, Libbey JE, Fujinami RS. Molecular mimicry as a mechanism of autoimmune disease. Clin Rev Allergy Immunol. 2012;42(1):102-11.

170. Clayton GM, Wang Y, Crawford F, Novikov A, Wimberly BT, Kieft JS, et al. Structural basis of chronic beryllium disease: linking allergic hypersensitivity and autoimmunity. Cell. 2014;158(1):132-42.

171. Newman LS. Metals that cause sarcoidosis. Semin Respir Infect. 1998;13(3):212-20.

172. Pavan C, Fubini B. Unveiling the Variability of "Quartz Hazard" in Light of Recent Toxicological Findings. Chem Res Toxicol. 2017;30(1):469-85.

173. Leung CC, Yu IT, Chen W. Silicosis. Lancet. 2012;379(9830):2008-18.

174. Calvert GM, Rice FL, Boiano JM, Sheehy JW, Sanderson WT. Occupational silica exposure and risk of various diseases: an analysis using death certificates from 27 states of the United States. Occup Environ Med. 2003;60(2):122-9.

175. Rafnsson V, Ingimarsson O, Hjalmarsson I, Gunnarsdottir H. Association between exposure to crystalline silica and risk of sarcoidosis. Occup Environ Med. 1998;55(10):657-60.

176. Falta MT, Pinilla C, Mack DG, Tinega AN, Crawford F, Giulianotti M, et al. Identification of beryllium-dependent peptides recognized by CD4+ T cells in chronic beryllium disease. J Exp Med. 2013;210(7):1403-18.

177. Bowerman NA, Falta MT, Mack DG, Wehrmann F, Crawford F, Mroz MM, et al. Identification of multiple public TCR repertoires in chronic beryllium disease. J Immunol. 2014;192(10):4571-80.

178. Dai S, Falta MT, Bowerman NA, McKee AS, Fontenot AP. T cell recognition of beryllium. Curr Opin Immunol. 2013;25(6):775-80.

179. Petukh M, Wu B, Stefl S, Smith N, Hyde-Volpe D, Wang L, et al. Chronic Beryllium Disease: revealing the role of beryllium ion and small peptides binding to HLA-DP2. PLoS One. 2014;9(11):e111604.

180. 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.

181. Darlington P, Haugom-Olsen H, von Sivers K, Wahlstrom J, Runold M, Svjatoha V, et al. T-cell phenotypes in bronchoalveolar lavage fluid, blood and lymph nodes in pulmonary sarcoidosis--indication for an airborne antigen as the triggering factor in sarcoidosis. J Intern Med. 2012;272(5):465-71.

182. 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.

183. 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.

184. 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.

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. Bronke C, Jansen CA, Westerlaken GH, De Cuyper IM, Miedema F, Tesselaar K, et al. Shift of CMV-specific CD4+ T-cells to the highly differentiated CD45RO-CD27- phenotype parallels loss of proliferative capacity and precedes progression to HIV-related CMV end-organ disease. Clin Immunol. 2007;124(2):190-9.

187. Park CO, Kupper TS. The emerging role of resident memory T cells in protective immunity and inflammatory disease. Nat Med. 2015;21(7):688-97.

188. 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.

189. Shigehara K, Shijubo N, Ohmichi M, Takahashi R, Kon S, Okamura H, et al. IL-12 and IL-18 are increased and stimulate IFN-gamma production in sarcoid lungs. J Immunol. 2001;166(1):642-9.

190. Antoniou KM, Tzouvelekis A, Alexandrakis MG, Tsiligianni I, Tzanakis N, Sfiridaki K, et al. Upregulation of Th1 cytokine profile (IL-12, IL-18) in bronchoalveolar lavage fluid in patients with pulmonary sarcoidosis. J Interferon Cytokine Res. 2006;26(6):400-5.

191. 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.

192. Ostadkarampour M, Eklund A, Moller D, Glader P, Olgart Hoglund C, Linden A, et al. Higher levels of interleukin IL-17 and antigen-specific IL-17 responses in pulmonary sarcoidosis patients with Lofgren's syndrome. Clin Exp Immunol. 2014;178(2):342-52.

193. Ramstein J, Broos CE, Simpson LJ, Ansel KM, Sun SA, Ho ME, et al. Interferon-gamma-producing Th17.1 Cells are Increased in Sarcoidosis and More Prevalent Than Th1 Cells. Am J Respir Crit Care Med. 2015.

194. Ramesh R, Kozhaya L, McKevitt K, Djuretic IM, Carlson TJ, Quintero MA, et al. Pro-inflammatory human Th17 cells selectively express P-glycoprotein and are refractory to glucocorticoids. J Exp Med. 2014;211(1):89-104.

195. Broos CE, Hendriks RW, Kool M. T-cell immunology in sarcoidosis: Disruption of a delicate balance between helper and regulatory T-cells. Curr Opin Pulm Med. 2016;22(5):476-83.

196. Ueda-Hayakawa I, Tanimura H, Osawa M, Iwasaka H, Ohe S, Yamazaki F, et al. Elevated serum BAFF levels in patients with sarcoidosis: association with disease activity. Rheumatology (Oxford). 2013;52(9):1658-66.

197. Saussine A, Tazi A, Feuillet S, Rybojad M, Juillard C, Bergeron A, et al. Active chronic sarcoidosis is characterized by increased transitional blood B cells, increased IL-10-producing regulatory B cells and high BAFF levels. PLoS One. 2012;7(8):e43588.

198. Lai Kwan Lam Q, King Hung Ko O, Zheng BJ, Lu L. Local BAFF gene silencing suppresses Th17-cell generation and ameliorates autoimmune arthritis. Proc Natl Acad Sci U S A. 2008;105(39):14993-8.

199. 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.

200. Sakthivel P, Grunewald J, Eklund A, Bruder D, Wahlstrom J. Pulmonary sarcoidosis is associated with high-level inducible co-stimulator (ICOS) expression on lung regulatory T cells--possible implications for the

ICOS/ICOS-ligand axis in disease course and resolution. Clin Exp Immunol. 2016;183(2):294-306.

201. 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.
202. Broos CE, van Nimwegen M, Kleinjan A, ten Berge B, Muskens F, in 't Veen JC, et al. Impaired survival of regulatory T cells in pulmonary sarcoidosis. Respir Res. 2015;16:108.
203. Miyara M, Amoura Z, Parizot C, Badoual C, Dorgham K, Trad S, et al. The immune paradox of sarcoidosis and regulatory T cells. J Exp Med. 2006;203(2):359-70.
204. Taflin C, Miyara M, Nochy D, Valeyre D, Naccache JM, Altare F, et al. FoxP3+ regulatory T cells suppress early stages of granuloma formation but have little impact on sarcoidosis lesions. Am J Pathol. 2009;174(2):497-508.
205. Rappl G, Pabst S, Riemann D, Schmidt A, Wickenhauser C, Schutte W, et al. Regulatory T cells with reduced repressor capacities are extensively amplified in pulmonary sarcoid lesions and sustain granuloma formation. Clin Immunol. 2011;140(1):71-83.
206. Palmer BE, Mack DG, Martin AK, Gillespie M, Mroz MM, Maier LA, et al. Up-regulation of programmed death-1 expression on beryllium-specific CD4+ T cells in chronic beryllium disease. J Immunol. 2008;180(4):2704-12.
207. Chain JL, Martin AK, Mack DG, Maier LA, Palmer BE, Fontenot AP. Impaired function of CTLA-4 in the lungs of patients with chronic beryllium disease contributes to persistent inflammation. J Immunol. 2013;191(4):1648-56.
208. Eklund A, du Bois RM. Approaches to the treatment of some of the troublesome manifestations of sarcoidosis. J Intern Med. 2014;275(4):335-49.
209. Vorselaars ADM, Wuyts WA, Vorselaars VMM, Zanen P, Deneer VHM, Veltkamp M, et al. Methotrexate vs azathioprine in second-line therapy of sarcoidosis. Chest. 2013;144(3):805-12.
210. Baughman RP, Lower EE. Treatment of Sarcoidosis. Clin Rev Allergy Immunol. 2015;49(1):79-92.
211. Paramothayan NS, Lasserson TJ, Jones PW. Corticosteroids for pulmonary sarcoidosis. Cochrane Database Syst Rev. 2005(2):CD001114.
212. Grutters JC, van den Bosch JM. Corticosteroid treatment in sarcoidosis. Eur Respir J. 2006;28(3):627-36.
213. Schacke H, Docke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. Pharmacol Ther. 2002;96(1):23-43.
214. Cremers JP, Drent M, Bast A, Shigemitsu H, Baughman RP, Valeyre D, et al. Multinational evidence-based World Association of Sarcoidosis and Other Granulomatous Disorders recommendations for the use of methotrexate in sarcoidosis: integrating systematic literature research and expert opinion of sarcoidologists worldwide. Curr Opin Pulm Med. 2013;19(5):545-61.

215. Lower EE, Baughman RP. Prolonged use of methotrexate for sarcoidosis. Arch Intern Med. 1995;155(8):846-51.

216. Lower EE, Baughman RP. The use of low dose methotrexate in refractory sarcoidosis. Am J Med Sci. 1990;299(3):153-7.

217. Baughman RP, Lower EE, Ingledue R, Kaufman AH. Management of ocular sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 2012;29(1):26-33.

218. Hoitsma E, Faber CG, Drent M, Sharma OP. Neurosarcoidosis: a clinical dilemma. Lancet Neurol. 2004;3(7):397-407.

219. 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.

220. Valeyre D, Jeny F, Nunes H. Current Medical Therapy for Sarcoidosis. Semin Respir Crit Care Med. 2017;38(4):523-31.

221. Baha A, Hanazay C, Kokturk N, Turktas H. A Case of Sarcoidosis Associated With Anti-Tumor Necrosis Factor Treatment. J Investig Med High Impact Case Rep. 2015;3(1):2324709615571366.

222. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens. 1992;39(5):225-35.

223. Olsen HH, Grunewald J, Tornling G, Skold CM, Eklund A. Bronchoalveolar lavage results are independent of season, age, gender and collection site. PLoS One. 2012;7(8):e43644.

224. Ranu H, Wilde M, Madden B. Pulmonary function tests. Ulster Med J. 2011;80(2):84-90.

225. Aaron SD, Dales RE, Cardinal P. How accurate is spirometry at predicting restrictive pulmonary impairment? Chest. 1999;115(3):869-73.

226. American Thoracic Society. Single-breath carbon monoxide diffusing capacity (transfer factor). Recommendations for a standard technique--1995 update. Am J Respir Crit Care Med. 1995;152(6 Pt 1):2185-98.

227. Grunewald J, Wahlstrom J, Berlin M, Wigzell H, Eklund A, Olerup O. Lung restricted T cell receptor AV2S3+ CD4+ T cell expansions in sarcoidosis patients with a shared HLA-DRbeta chain conformation. Thorax. 2002;57(4):348-52.

228. Ziegler SF, Ramsdell F, Alderson MR. The activation antigen CD69. Stem Cells. 1994;12(5):456-65.

229. Kumar BV, Ma W, Miron M, Granot T, Guyer RS, Carpenter DJ, et al. Human Tissue-Resident Memory T Cells Are Defined by Core Transcriptional and Functional Signatures in Lymphoid and Mucosal Sites. Cell Rep. 2017;20(12):2921-34.

230. Czerkinsky CC, Nilsson LA, Nygren H, Ouchterlony O, Tarkowski A. A solid-phase enzyme-linked immunospot (ELISPOT) assay for enumeration of specific antibody-secreting cells. J Immunol Methods. 1983;65(1-2):109-21.

231. Turchaninova MA, Britanova OV, Bolotin DA, Shugay M, Putintseva EV, Staroverov DB, et al. Pairing of T-cell receptor chains via emulsion PCR. Eur J Immunol. 2013;43(9):2507-15.

232. Bruggner RV, Bodenmiller B, Dill DL, Tibshirani RJ, Nolan GP. Automated identification of stratifying signatures in cellular subpopulations. Proc Natl Acad Sci U S A. 2014;111(26):E2770-7.

233. Shekhar K, Brodin P, Davis MM, Chakraborty AK. Automatic Classification of Cellular Expression by Nonlinear Stochastic Embedding (ACCENSE). Proc Natl Acad Sci U S A. 2014;111(1):202-7.

234. Chen H, Lau MC, Wong MT, Newell EW, Poidinger M, Chen J. Cytofkit: A Bioconductor Package for an Integrated Mass Cytometry Data Analysis Pipeline. PLoS Comput Biol. 2016;12(9):e1005112.

235. RStudio Team. RStudio: Integrated Development for R. 2015. Available from: http://www.rstudio.com/.

236. Olsen I, Sollid LM. Pitfalls in determining the cytokine profile of human T cells. J Immunol Methods. 2013;390(1-2):106-12.

237. Wei G, Wei L, Zhu J, Zang C, Hu-Li J, Yao Z, et al. Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. Immunity. 2009;30(1):155-67.

238. Schuh E, Berer K, Mulazzani M, Feil K, Meinl I, Lahm H, et al. Features of Human CD3+CD20+ T Cells. J Immunol. 2016;197(4):1111-7.

239. Lexberg MH, Taubner A, Albrecht I, Lepenies I, Richter A, Kamradt T, et al. IFN-gamma and IL-12 synergize to convert in vivo generated Th17 into Th1/Th17 cells. Eur J Immunol. 2010;40(11):3017-27.

240. Hueber W, Sands BE, Lewitzky S, Vandemeulebroecke M, Reinisch W, Higgins PD, et al. Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. Gut. 2012;61(12):1693-700.

241. Symons A, Budelsky AL, Towne JE. Are Th17 cells in the gut pathogenic or protective? Mucosal Immunol. 2012;5(1):4-6.

242. 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.

243. 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.

244. McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, McClanahan T, et al. TGF-beta and IL-6 drive the production of IL-17 and IL-

10 by T cells and restrain T(H)-17 cell-mediated pathology. Nat Immunol. 2007;8(12):1390-7.

245. Idali F, Wahlstrom J, Dahlberg B, Khademi M, Olsson T, Eklund A, et al. Altered expression of T cell immunoglobulin-mucin (TIM) molecules in bronchoalveolar lavage CD4+ T cells in sarcoidosis. Respir Res. 2009;10:42.

246. Braun NA, Celada LJ, Herazo-Maya JD, Abraham S, Shaginurova G, Sevin CM, et al. Blockade of the programmed death-1 pathway restores sarcoidosis CD4(+) T-cell proliferative capacity. Am J Respir Crit Care Med. 2014;190(5):560-71.

247. Celada LJ, Rotsinger JE, Young A, Shaginurova G, Shelton D, Hawkins C, et al. Programmed Death-1 Inhibition of Phosphatidylinositol 3-Kinase/AKT/Mechanistic Target of Rapamycin Signaling Impairs Sarcoidosis CD4(+) T Cell Proliferation. Am J Respir Cell Mol Biol. 2017;56(1):74-82.

248. Batista MD, Tincati C, Milush JM, Ho EL, Ndhlovu LC, York VA, et al. CD57 expression and cytokine production by T cells in lesional and unaffected skin from patients with psoriasis. PLoS One. 2013;8(2):e52144.

249. Fernandez S, French MA, Price P. Immunosenescent CD57+CD4+ T-cells accumulate and contribute to interferon-gamma responses in HIV patients responding stably to ART. Dis Markers. 2011;31(6):337-42.

250. Palmer BE, Blyveis N, Fontenot AP, Wilson CC. Functional and phenotypic characterization of CD57+CD4+ T cells and their association with HIV-1-induced T cell dysfunction. J Immunol. 2005;175(12):8415-23.

251. Appay V, Zaunders JJ, Papagno L, Sutton J, Jaramillo A, Waters A, et al. Characterization of CD4(+) CTLs ex vivo. J Immunol. 2002;168(11):5954-8.

252. Parasa VR, Forsslund H, Enger T, Lorenz D, Kullberg S, Eklund A, et al. Enhanced CD8+ cytolytic T cell responses in the peripheral circulation of patients with sarcoidosis and non-Lofgren's disease. Respir Med. 2017.

253. Kamphuis LS, van Zelm MC, Lam KH, Rimmelzwaan GF, Baarsma GS, Dik WA, et al. Perigranuloma localization and abnormal maturation of B cells: emerging key players in sarcoidosis? Am J Respir Crit Care Med. 2013;187(4):406-16.

254. Hunninghake GW, Crystal RG. Mechanisms of hypergammaglobulinemia in pulmonary sarcoidosis. Site of increased antibody production and role of T lymphocytes. J Clin Invest. 1981;67(1):86-92.

255. Duhlin A, Chen Y, Wermeling F, Sedimbi SK, Lindh E, Shinde R, et al. Selective Memory to Apoptotic Cell-Derived Self-Antigens with Implications for Systemic Lupus Erythematosus Development. J Immunol. 2016;197(7):2618-26.

256. Onuora S. Autoimmunity: Apoptotic cells induce immune memory. Nat Rev Rheumatol. 2016;12(10):559.

257. Jones JV, Cumming RH, Asplin CM. Evidence for circulating immune complexes in erythema nodosum and early sarcoidosis. Ann N Y Acad Sci. 1976;278:212-9.

258. Ikehata A, Tomichi N. CD44v6 expression in granuloma of Crohn's disease. Am J Gastroenterol. 2000;95(11):3325-6.

259. Ohyashiki K, Ando K, Hayashi S, Ohyashiki JH, Hara A, Nasu H, et al. Soluble CD44 level in non-malignant disorders is associated with autoimmune backgrounds. Autoimmunity. 1999;30(1):35-6.

260. Kasuga I, Minemura K, Nasu H, Hibi N, Tago H, Nagate A, et al. Elevated serum soluble CD44 level in sarcoidosis. Int J Mol Med. 2000;6(6):679-82.

261. Linke M, Pham HT, Katholnig K, Schnoller T, Miller A, Demel F, et al. Chronic signaling via the metabolic checkpoint kinase mTORC1 induces macrophage granuloma formation and marks sarcoidosis progression. Nat Immunol. 2017.

262. Gadhoum SZ, Madhoun NY, Abuelela AF, Merzaban JS. Anti-CD44 antibodies inhibit both mTORC1 and mTORC2: a new rationale supporting CD44-induced AML differentiation therapy. Leukemia. 2016;30(12):2397-401.

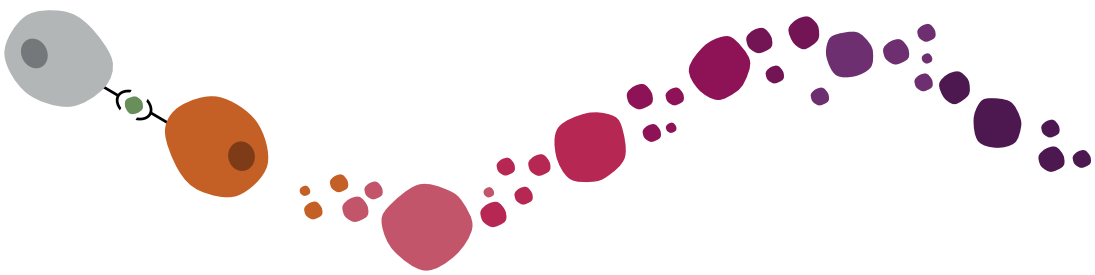
263. Dorum S, Steinsbo O, Bergseng E, Arntzen MO, de Souza GA, Sollid LM. Gluten-specific antibodies of celiac disease gut plasma cells recognize long proteolytic fragments that typically harbor T-cell epitopes. Sci Rep. 2016;6:25565.

264. Mor-Vaknin N, Punturieri A, Sitwala K, Markovitz DM. Vimentin is secreted by activated macrophages. Nat Cell Biol. 2003;5(1):59-63.

265. Li FJ, Surolia R, Li H, Wang Z, Kulkarni T, Liu G, et al. Autoimmunity to Vimentin Is Associated with Outcomes of Patients with Idiopathic Pulmonary Fibrosis. J Immunol. 2017;199(5):1596-605.

266. Nagvekar N, Corlett L, Jacobson LW, Matsuo H, Chalkley R, Driscoll PC, et al. Scanning a DRB3*0101 (DR52a)-restricted epitope cross-presented by DR3: overlapping natural and artificial determinants in the human acetylcholine receptor. J Immunol. 1999;162(7):4079-87.

267. Banks SJ, Sreenivasan KR, Weintraub DM, Baldock D, Noback M, Pierce ME, et al. Structural and Functional MRI Differences in Master Sommeliers: A Pilot Study on Expertise in the Brain. Front Hum Neurosci. 2016;10:414.



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