Autoimmune disease – phenotypic alterations of peripheral immune cells and clinical correlates

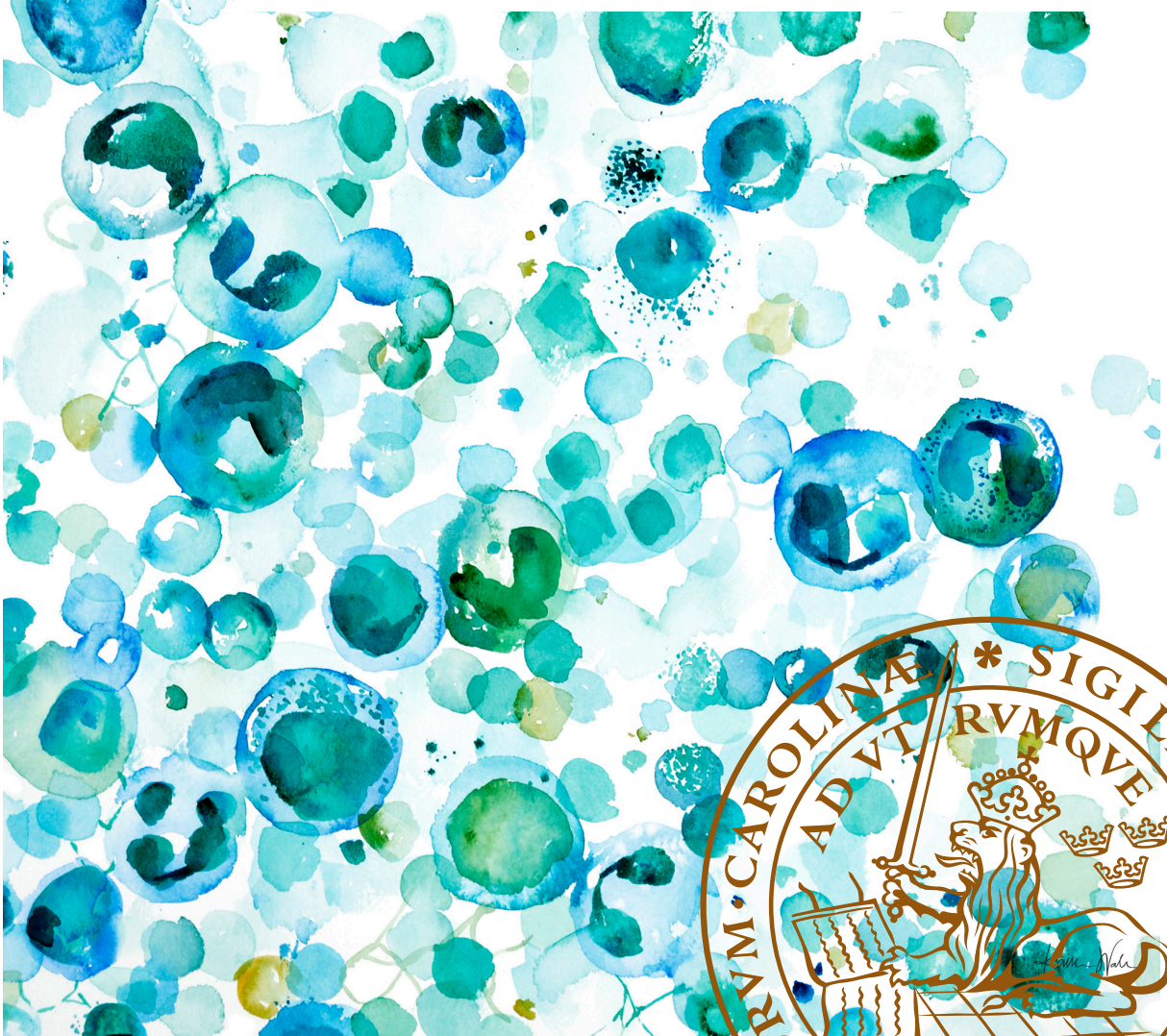
Elmér, Evelina

2022

Document Version:
Publisher's PDF, also known as Version of record

Autoimmune disease – phenotypic alterations of peripheral immune cells and clinical correlates

EVELINA ELMÉR
DEPARTMENT OF LABORATORY MEDICINE | FACULTY OF MEDICINE | LUND UNIVERSITY



Autoimmune disease - phenotypic alterations of peripheral immune cells and clinical correlates

Autoimmune disease - phenotypic alterations of peripheral immune cells and clinical correlates

Evelina Elmér

LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on October 28, 2022, at 09.00 in Lecture Hall 4, Blocket, Skåne University Hospital, Lund

Faculty opponent
Associate professor Mats Bemark
Department of Microbiology and Immunology, University of Gothenburg, Sweden

Organization LUND UNIVERSITY Department of Laboratory Medicine, Division of Hematology and Transfusion Medicine	**Document name** DOCTORAL DISSERTATION **Date of issue** October 28, 2022
Author Evelina Elmér	Sponsoring organization

Title
Autoimmune disease - phenotypic alterations of peripheral immune cells and clinical correlates

Abstract

This thesis comprises research projects related to alterations of peripheral immune cells in patients with autoimmune disease, and sequence variants associated with immunoglobulin levels in the general population.

In paper I, the genetic basis of variability in immunoglobulin levels in the general population was explored in a genome-wide association study of 19 219 individuals. Thirty-eight new and five known sequence variants associating with IgA, IgG or IgM levels or with composite immunoglobulin traits were found. The results provide new insight into the regulation of humoral immunity.

In paper II, we showed that patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) exhibit decreased frequencies of B cells and transitional B cells, as well as higher proportions of switched memory B cells, plasmablasts and activated B cells in peripheral blood. Further, there were differences in B cell subset frequencies between relapsing and nonrelapsing patients. These alterations could contribute to the autoantibody-driven inflammatory process in AAV.

In paper III, we investigated the role of granulocytes and monocytes in the faltering antibody response following immunization with pneumococcal conjugate vaccine in rheumatoid arthritis patients treated with methotrexate. Following methotrexate treatment, the frequency and concentration of monocytes were lower in future nonresponders to the vaccine, constituting a potential biomarker of the antibody response in this patient group.

In paper IV, we demonstrated that AAV patients in active disease display increased concentration of mature neutrophils and decreased percentage of monocytes. Patients with a tendency to relapse presented increased frequencies of mature ($CD16^{high}$) and $CD177^{+}$ neutrophils. These changes may be used for relapse prediction in this patient group.

Key words ANCA-associated vasculitis, immunization, relapse, rheumatoid arthritis, sequence variant		
Classification system and/or index terms		
Supplementary bibliographical information		**Language** English
ISSN and key title 1652-8220		**ISBN** 978-91-8021-299-1
Recipient's notes	**Number of pages** 76	Price
	Security classification	

Signature  Date 2022-09-22

Autoimmune disease - phenotypic alterations of peripheral immune cells and clinical correlates

Evelina Elmér

LUND
UNIVERSITY

Cover illustration by Kaitlin Walsh

Faculty of Medicine
Department of Laboratory Medicine

ISBN 978-91-8021-299-1
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2022

To my family

Contents

List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Jonsson S, Sveinbjornsson G, de Lapuente Portilla AL, Swaminathan B, Plomp R, Dekkers G, Ajore R, Ali M, Bentlage AEH, **Elmér E**, Eyjolfsson GI, Gudjonsson SA, Gullberg U, Gylfason A, Halldorsson BV, Hansson M, Holm H, Johansson Å, Johnsson E, Jonasdottir A, Ludviksson BR, Oddsson A, Olafsson I, Olafsson S, Sigurdardottir O, Sigurdsson A, Stefansdottir L, Masson G, Sulem P, Wuhrer M, Wihlborg AK, Thorleifsson G, Gudbjartsson DF, Thorsteinsdottir U, Vidarsson G, Jonsdottir I, Nilsson B, Stefansson K. Identification of sequence variants influencing immunoglobulin levels. *Nature Genetics* 2017;49(8):1182-91

II. **Elmér E**, Smargianaki S, Pettersson Å, Skattum L, Ohlsson S, Hellmark T, Johansson Å CM. Increased frequencies of switched memory B cells and plasmablasts in peripheral blood from patients with ANCA-associated vasculitis. *Journal of Immunology Research* 2020:8209737

III. **Elmér E**, Nived P, Pettersson Å, Skattum L, Hellmark T, Kapetanovic MC, Johansson Å CM. Methotrexate treatment suppresses monocytes in nonresponders to pneumococcal conjugate vaccine in rheumatoid arthritis patients. *Journal of Immunology Research* 2022:7561661

IV. Smargianaki S, **Elmér E**, Lilliebladh S, Ohlsson S, Pettersson Å, Hellmark T, Johansson Å CM. Disease activity and tendency to relapse in ANCA-associated vasculitis are reflected in neutrophil and intermediate monocyte frequencies. *Manuscript*

Abbreviations

AAV	ANCA-associated vasculitis
ANCA	anti-neutrophil cytoplasmic antibody
APC	antigen presenting cell
BCR	B cell receptor
CD	cluster of differentiation
CTLA-4	cytotoxic T lymphocyte-associated protein 4
DC	dendritic cell
DMARD	disease-modifying antirheumatic drug
GC	germinal center
GWAS	genome-wide association study
HLA	human leukocyte antigen
IFN-γ	interferon gamma
Ig	immunoglobulin
IL	interleukin
MHC	major histocompatibility complex
MPO	myeloperoxidase
MTX	methotrexate
NET	neutrophil extracellular trap
NK	natural killer
PCV	pneumococcal conjugate vaccine
PD-1	programmed cell death protein 1
PR3	proteinase 3
RA	rheumatoid arthritis
ROS	reactive oxygen species
SHM	somatic hypermutation
Siglec	sialic acid binding immunoglobulin-like lectin
TCR	T cell receptor
Tfh cell	follicular T helper cell
TGF-β	transforming growth factor beta
Th cell	T helper cell
TNF-α	tumour necrosis factor alpha
Treg	regulatory T cell

Introduction

Innate and adaptive immunity

In humans, a large number of immunological mechanisms are capable of destroying pathogenic organisms such as bacteria, fungi, viruses, and parasites. The immune system comprises two interrelated systems, the innate, present at birth, and the adaptive, which is acquired and continues to evolve throughout life. Defects in either can cause disorders such as autoimmune disease, immunodeficiency and hypersensitivity reactions.

Innate immunity is the first line of defence against pathogens and possesses essential protective functions against microbes and tissue injuries. Innate immunity includes physical, chemical and biological barriers, the complement system and innate immune cells and their effector molecules. It is triggered immediately or within hours of antigen exposure. The innate immune response has no immunologic memory. There is no refinement of the response during the infection and no enhancement upon repeated exposure. Innate immune cells do not have the same antigen specificity as the adaptive immunity but do have defined selectivity against pathogens. By recognizing germline-encoded microbial structures known as pathogen-associated molecular patterns (PAMPs) and endogenous products of cell damage known as damage-associated molecular patterns (DAMPs), via pattern recognition receptors (PRRs), defence mechanisms are triggered regardless of the specific nature of the microbe.

Invading pathogens, foreign molecules, damaged or dead cells unrelated to infection, activate the innate immune system. Importantly, activation of innate immunity triggers rapid recruitment of immune cells to the sites of infection, vascular permeability changes and release of inflammatory mediators, causing inflammation. The primary purpose of the inflammation is to contain the infection and promote healing once the pathogens have been cleared. In the affected tissue, inflammation is characterized by redness, swelling, heat, pain and loss of function. In addition to local cellular responses to infection or injury, cytokine release mobilizes different defence mechanisms throughout the body, including contributing to the development of fever.

The effector responses of the innate immune system are numerous and include production of reactive oxygen species (ROS) and reactive nitrogen species (RNS),

release of proteolytic and bacteriostatic peptides, production of cytokines, clearance of immune complexes and cellular debris, direct cell killing via death receptors, and phagocytosis.

Broadly, innate immune cells originate from myeloid progenitors and adaptive immune cells from lymphoid progenitors (Figure 1). Innate immune cells include granulocytes (polymorphonuclear cells), monocytes, macrophages, dendritic cells (DC), mast cells, natural killer (NK) cells and innate lymphoid cells (ILCs).

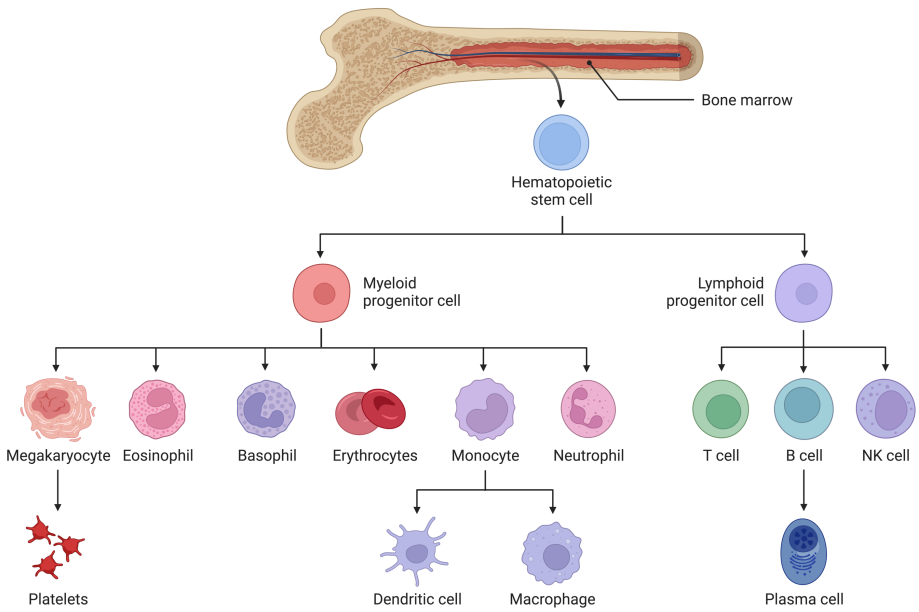


Figure 1. Stem cell differentiation from bone marrow. Reprinted from BioRender.com (2022).

The adaptive immune system displays receptor-mediated specificity for a particular pathogen, toxin or allergen - distinguishing them from "self" antigens. The responding cells must proliferate after encountering a foreign antigen in order to elicit an effective response. The response is therefore generally delayed relative to innate immunity but can, on the other hand, accelerate during an infection. A key feature of the adaptive response is the production of long-lived memory cells that can rekindle effector functions rapidly after another encounter with the specific antigen, even decades after the initial exposure.

The key elements of the adaptive immune system are the lymphocytes, T and B cells, together with immunoglobulins. T and B cells were named after their initial characterization in the thymus and bursa of Fabricius, respectively[1]. The bursa of

Fabricius, where the B cells differentiate, is unique to birds. In humans, T and B cells are formed in the bone marrow, and mature in thymus and bone marrow, respectively. T cells have a central role in regulating the immune response and are responsible for the cell-mediated immunity, whilst B cells are crucial in the effector phase of humoral immunity by producing antibodies, but also have other functions.

Innate immune cells

Numerous cell types are involved in the innate immune response. Some of these cells and their main functions are as follows[2, 3].

Granulocytes

Granulocytes encompass three cell types; neutrophils, eosinophils and basophils, differentiated by the contents of their granules. They are all relatively short-lived cells.

Neutrophils

Neutrophils play a major role in the resolution of infections caused by bacteria and fungi. They make up about half of the circulating white blood cells in humans and are typically the first cells to arrive at the site of infection. Neutrophils exert antimicrobial actions through three main mechanisms: phagocytosis, degranulation, and formation of neutrophil extracellular traps (NETs). In chronic inflammation the role of neutrophils is less well understood, and both beneficial and detrimental effects have been proposed. In line with this, patients with inflammation exhibit a heterogeneous population of neutrophils. The population includes low-density neutrophils consisting of e.g. granulocytic/polymorphonuclear-myeloid-derived suppressor cells (PMN-MDSCs) with immunosuppressive properties and proinflammatory low-density granulocytes (LDGs). Neutrophils migrate to the site of chronic inflammation, release serine proteases and NETs, as well as activate other immune cells[4]. The neutrophils are thought to play an important role in the pathogenesis of anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and are found in and around inflamed vessel walls. ANCAs activate neutrophils leading to degranulation, production of ROS, complement activation and release of NETs[5, 6]. Mature neutrophils are $CD10^{+}CD16^{high}$, whereas increased $CD10^{-}CD16^{dim}$ cells in peripheral blood are thought to reflect an augmented mobilization of neutrophils from the bone marrow. AAV patients have an increased frequency of $CD10^{-}CD16^{dim}$ and mature neutrophils in peripheral blood, suggesting a combination of increased bone marrow release and prolonged survival[7].

Eosinophils

Eosinophils play a significant role in the clearance of parasites and in inflammatory processes associated with allergy and asthma. At the inflammation site, eosinophils

can modify the immune response via secretion of proinflammatory mediators. Eosinophils can also act as effector cells and induce tissue damage by releasing the cytotoxic content of their granules. The granules contain mainly four proteins that are directly related to the eosinophil's effector functions: major basic protein, eosinophil cationic protein, eosinophil peroxidase, and neurotoxin derived from eosinophils. In addition, eosinophils can initiate antigen-specific immune responses by acting as antigen presenting cells (APCs)[8].

Basophils

Basophils are the least abundant leukocyte in blood (<1%). These cells are important in all allergic diseases and can induce and propagate Th2 immune responses. When activated by antigen cross-linking of FcεRI-bound IgE, basophils rapidly release histamine and synthesize leukotriene C4, followed by production of Th2 cytokines such as IL-4 and IL-13, causing allergic symptoms. Basophils can also be activated without IgE crosslinking e.g. by complement factors and chemokines, and are considered particularly important for the immune response against parasites.

Mast cells

Mast cells are long-lived cells with an important role in the immune response against parasites and in allergic reactions. Mast cells reside in tissues in contact with the external environment, e.g. at mucosal surfaces of the lungs and gut, in the skin and around blood vessels. Similar to circulating basophils, mast cells express FcεRI and promotes Th2 lymphocyte differentiation and IgE production. Cross-linking of the FcεRI by IgE-antigens induces mast cell activation and rapid release of proinflammatory mediators (e.g. histamine, TNF-α, IL-4, IL-13) via degranulation.

Monocytes

Monocytes are short-lived circulating mononuclear phagocytes, and precursors of tissue macrophages and monocyte-derived dendritic cells. They comprise 10-15% of human peripheral blood mononuclear cells (PBMCs) and react to infection with tissue migration and cytokine production, and can also act as APCs to T cells. Depending on the condition, monocytes can act either proinflammatory via cytokine release and antigen presentation, or anti-inflammatory via promotion of regulatory T cells and suppression of T cell proliferation[9, 10]. Monocytes are usually divided into three phenotypically and functionally distinct subgroups based on their expression of lipopolysaccharide (LPS) receptor, CD14, and FcγRIII, CD16[11]. Classical monocytes express high levels of CD14 but no CD16 and comprise 80-95% of the monocyte pool. They display high phagocytic capacity, proinflammatory properties and antimicrobial effects. Intermediate monocytes show high levels of CD14 and low CD16, and comprise 2-8% of circulating monocytes. They participate in the proliferation and stimulation of T cells, antigen presentation, production of ROS and are also implicated in angiogenesis. Non-classical

monocytes express low levels of CD14 and high CD16, and comprise 2-11% of peripheral monocytes. They contribute to antiviral responses, patrol and maintain endothelial homeostasis and can act proinflammatory[12, 13]. Further, several inflammatory diseases such as rheumatoid arthritis and ANCA-associated vasculitis are associated with an increased population of intermediate monocytes in blood[14, 15].

Dendritic cells

Dendritic cells (DCs) are present in practically all tissues and encompass one-tenth of leukocytes in human blood. Human DCs can be identified by high expression of major histocompatibility complex (MHC) class II and CD11c, and have been classified into various subtypes including classical (cDC1/cDC2), monocyte-derived, plasmacytoid DCs etc. based on phenotypic and functional attributes, which recently have been questioned[16]. DCs detect cell damage signals, microbial antigens and foreign molecules using surface receptors including TLRs, and capture antigens. Upon stimulation they become activated (mature), and quickly migrate to the draining lymph nodes to present antigens and convey signals, primarily to T cells but also to B cells[17], and interact with other immune and non-immune cells[18]. Further, DCs play an important role by presenting self-antigens to $CD4^+$ and $CD8^+$ T cells, maintaining immune tolerance[16].

Macrophages

Tissue-resident macrophages (TRM) originate either from embryonic progenitors or circulating monocytes, and have a wide distribution throughout the body, e.g. lung, liver (Kupffer cells), spleen and brain (microglia). Macrophages are heterogenous in their morphology and functional features (e.g. surface receptors, pathogen selectivity and cytokine release), largely depending on the tissue environment in which they reside. TRMs are classically polarized into proinflammatory macrophages (M1) by cytokines such as IFN-γ and TNF-α, alone or with lipopolysaccharide (LPS) from bacteria, alternatively activated into anti-inflammatory macrophages (M2) by IL-4/IL-13. M1-related functions promote the Th1 immune response via cytokine release, phagocytosis and destruction of pathogens or damaged/tumorous cells[19], and as well antigen presentation to stimulate adaptive immunity. M2-related macrophage functions promote the Th2 immune response by antagonizing the inflammatory response. However, the M1/M2 classification does not cover all states of macrophage activation[20].

Innate lymphoid cells

Innate lymphoid cells (ILCs) are non-T, non-B lymphocytes that have recently been classified into five subsets based on various phenotypic and functional features: natural killer (NK) cells, ILC1, ILC2, ILC3, and lymphoid tissue-inducer cells (LTi). Their functions largely overlap with that of T cells and could be considered

their innate counterparts. Each subset releases a selection of cytokines and mediators, depending on their actions, e.g. NK cells can rapidly recognize the absence of cell surface MHC and kill cancerous or virally infected cells via release of lytic granules containing perforin and granzymes. In addition, NK cells (as well as ILC1 cells) produce IFN-γ and contribute to the adaptive immunity by triggering T cell–mediated responses. Most ILCs reside in mucosal tissues and display a large array of immune functions including regulation of inflammation and innate immune responses to different pathogens, such as intracellular microbes and cancer cells (NK cells and ILC1), large parasites and allergens (ILC2) and extracellular microbes (ILC3). LTi cells are important for embryonic lymph node formation[21].

Non-typical innate immune cells

Natural killer T (NKT) cells are lymphocytes that express both T cell receptor (TCR) and NK surface receptors. They have the ability to respond to cells participating in innate as well as adaptive immunity. NKT cells participate in surveillance of tumours, maintenance of self-tolerance and have been implicated in the regulation of autoimmune diseases[22].

Gamma delta (γδ) T cells are a group of "unconventional" T cells. They only account for 1-5% of T cells in peripheral blood, with highest abundance in the gut mucosa[23]. γδ T cells are defined by TCRs composed of γ and δ chains ("conventional" T cells express αβ TCRs) and display both innate- and adaptive-like properties. They may be considered innate immune cells in that their TCRs do not require MHC-mediated antigen presentation. Further, they have the ability to recognize antigens via germline-encoded regions of the receptor, reminiscent of PRR[24].

Cell-mediated immunity

Cell-mediated immunity is a term that emerged, historically, due to the inability to transfer immunity between animals simply by administration of antibody-containing plasma, suggesting a cellular base for immunity.

T cells are formed in the bone marrow and mature in the thymus. Naive T cells recirculate between blood and peripheral lymphoid tissue until they encounter their specific antigen in the form of a peptide-MHC complex on the surface of activated professional APCs, such as cells of the innate immune system, but also B cells. To recognize antigens, the T cell holds antigen-receptor molecules on its surface, termed T cell receptor (TCR), which consists of one α and one β chain (each consisting of two extracellular domains, a variable and a constant region), linked by a disulfide bond. The TCR αβ heterodimer (responsible for antigen recognition) is associated to CD3 (composed of four distinct chains), required for intracellular signalling, forming the TRC-CD3 complex (Figure 3). There are also T cells with

alternative chains in their TCR designated γ and δ, resulting in different properties. The TCR structure is very similar to the immunoglobulin molecule but has only one antigen-binding site, and the receptors are never secreted. During development of the T cell, the genes for the TCR are rearranged by a process called V(D)J recombination, producing an enormous variation in antigen receptors. It is produced by double strand DNA breaks and subsequent repair[25, 26]. DNA cleavage is executed by a recombinase complex consisting of the proteins RAG1 and RAG2, while the repair is produced by classical non-homologous end joining proteins[27]. Additional genetic variability is conferred by random insertion of nucleotides between the gene segments (junctional diversity). V(D)J recombination is unique to lymphocytes.

When T cells migrate to the thymus, they acquire the expression of both CD4 and CD8. Cells recognizing MHC class I lose CD4, and those recognizing MHC class II lose CD8. This results in two major subsets of T cells, CD4 T cells and CD8 T cells. Naive T cells transform to a variety of effector T cells when they come into contact with their specific antigen[28]. Effector T cells detect processed peptide antigens from different types of pathogens. Peptides from intracellular pathogens such as viruses and cancer cells, associate with MHC class I (expressed on nucleated cells) and are presented to CD8 T cells, which matures into cytotoxic T cells. The major function of these cells is destruction of infected cells through targeted release of granules containing perforin and granzymes, or by expression of CD154 (Fas ligand, FasL) on the T cell surface that engages CD95 (Fas) on the target cell, inducing apoptosis. Peptide antigens derived from ingested extracellular bacteria and toxins are transferred to the cell surface, associated with MHC class II (expressed on B cells, macrophages and DCs) and presented to CD4 T cells. T cell proliferation and cytokine release are directed by both TCR binding to the antigen peptide-MHC complex, and by interactions between cell-surface receptors on the T cells and their ligands on the APC. For example, CD28 on the T cell interacts with CD80 or CD86 on the APC, enhancing the activation of the T cell. While others, e.g. CTLA-4 and PD-1 on the T cell, and their ligands on APC inhibit T cell activation. Following antigen recognition, the responding T cells undergo clonal expansion, largely driven by IL-2 secreted by the T cells themselves.

When stimulated, naive CD4 T cells differentiate towards a Th1, Th2, Th17, follicular T helper (Tfh), or regulatory T cell (Treg) phenotype[29, 30] (Figure 2). New CD4 T cell subsets have been proposed, such as Th3, Tr1, Th9 and Th22[31]. The subsets express specific cytokines and transcription factors; Th1 (IFN-γ and T-bet), Th2 (IL-4, IL-5, IL-13 and GATA3), Th17 (IL-17, IL-22 and RORγt), Tfh (IL-21 and Bcl6) and Treg (IL-10, TGF-β, IL-35 and Foxp3)[31]. Th1 cells participate in immune responses to intracellular pathogens such as viruses, Th2 cells to larger extracellular pathogens such as helminths, Th17 cells to extracellular pathogens including bacteria and fungi, and are also associated with autoimmunity, Tfh cells provide help to B cells, and Treg cells, regulate immune responses by e.g. maintaining tolerance to self-antigens. Cytotoxic CD4 T cells (CD4-CTL) constitute

a recently identified CD4 subset with cytotoxic function. Similar to CD8 T cells, they kill target cells via secretion of cytotoxic granules in an antigen-specific fashion upon direct contact[32].

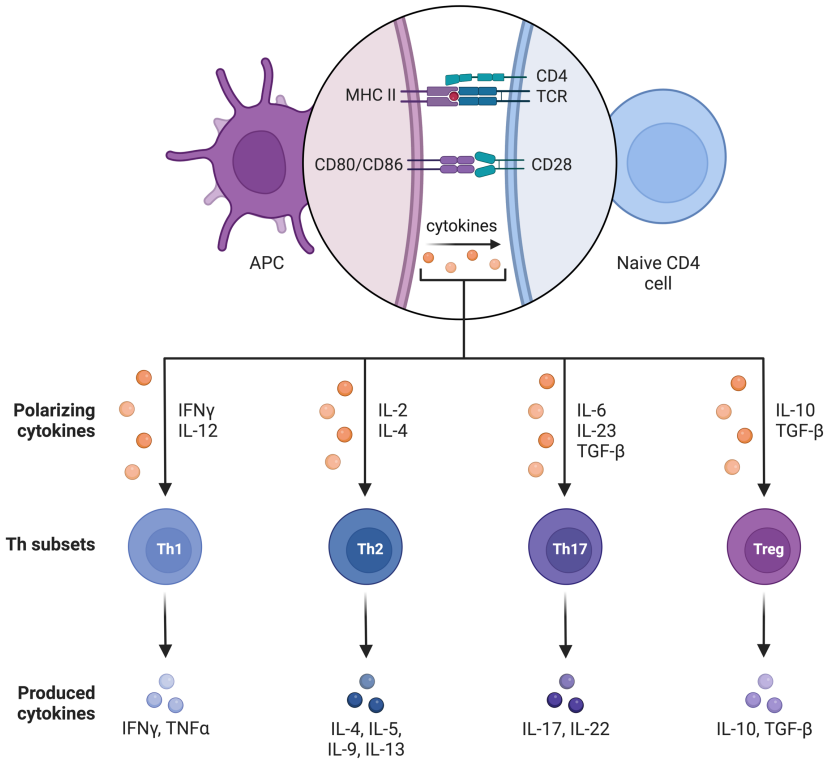


Figure 2. T cell activation and differentiation. Signal 1 (binding of the T cell receptor (TCR) to the peptide-MHCII complex on the antigen-presenting cell (APC) surface) and signal 2 (binding of the T cell co-receptor CD28 to CD80/CD86 on the APC surface) are required for T cell activation. The binding of polarizing cytokines to their respective receptor on the T cell surface represents signal 3. Different combinations of these cytokines influence T cell differentiation into distinct effector T cell subtypes that produce signature cytokines. Reprinted from BioRender.com (2022).

When effector T cells have reached the site of infection, they usually stop dividing. Since CD8 T cells can express both Fas and FasL molecules, the Fas/FasL interaction also leads to elimination of CD8 T cells at the end of an immune response. T cells can give rise to memory cells that have improved effectiveness upon re-exposure to the same antigen. Memory cells can persist for many years, although require cytokines, such as IL-5, to continue dividing every few months.

Humoral immunity

The origin of humoral immunity can be traced to vertebrates more than 500 million years ago[33]. B cells, in addition to the complement system, are at the center of the adaptive humoral immune system by generating antibody responses[34]. As mentioned above, in the 1960s, Max Cooper demonstrated in animal experiments that antibody production was connected to the bursa of Fabricius (main organ of B cell development in birds) from which the term 'B' cell was hence derived. The main B cell populations detected in human peripheral blood include transitional B cells (T1/T2, T2-MZP, T3), naive B cells (resting, activated, anergic), memory B cells (unswitched, pre-switched, switched resting, switched activated, atypical tissue-based), double negative (DN1, DN2), regulatory B cells (Breg, several phenotypes are defined by surface markers and by specific interleukin production) and antibody secreting cells (early plasmablast, plasmablast, plasma cell), regulatory plasma cells (PCreg) and B1 cells[35]. Recently, Glass et al., by screening the expression of a very large number of surface molecules and functional readouts, proposed a new classification of twelve unique subsets of human B cells[36]. They further evaluated tissue B cell subset proportions and calculated dissimilarity of tissues. The lymphoid tissue was most similar to peripheral blood, and peripheral blood was most similar to bone marrow. Only two subsets were not detected in peripheral blood: germinal center (GC) B cells and a $CD39^{+}$ tonsillar population.

The B cell receptor (BCR) consists of a membrane-bound immunoglobulin (Ig) and two signal-transducing subunits, Igα and Igβ (Figure 3). The membrane bound Ig:s (A, D, E, G, and M) contain two identical heavy polypeptide chains and two identical light chains. The variations in the constant regions of the heavy chains classify Ig molecules into classes (isotypes) and subclasses with different biological effects, while the variable regions determine the antigen specificity. Naive B cells express IgD and IgM, whereas memory B cells express IgA, IgG, or IgE[37]. Upon antigen binding, signal transduction will be conveyed by the Igα/β heterodimer, through their Ig-like extracellular domains and intracellular immunoreceptor tyrosine-based activation motifs (ITAMs)[38]. Owing to the genetic somatic recombination, specifically named V(D)J recombination, the BCR (like the T cell receptor described above) can display an almost limitless variation of potential antigen binding specificities[39]. In V(D)J recombination, exons that encode the antigen binding domains are constructed from three gene segments: V (variable), D (diversity), and J (joining). Double-strand breaks are induced at selected segments followed by deletion or inversion of DNA, and then end ligation[40]. The order of rearrangements of segments is controlled with D to J being joined before the V segment, significantly contributing to the antigen receptor diversity in the BCR[39].

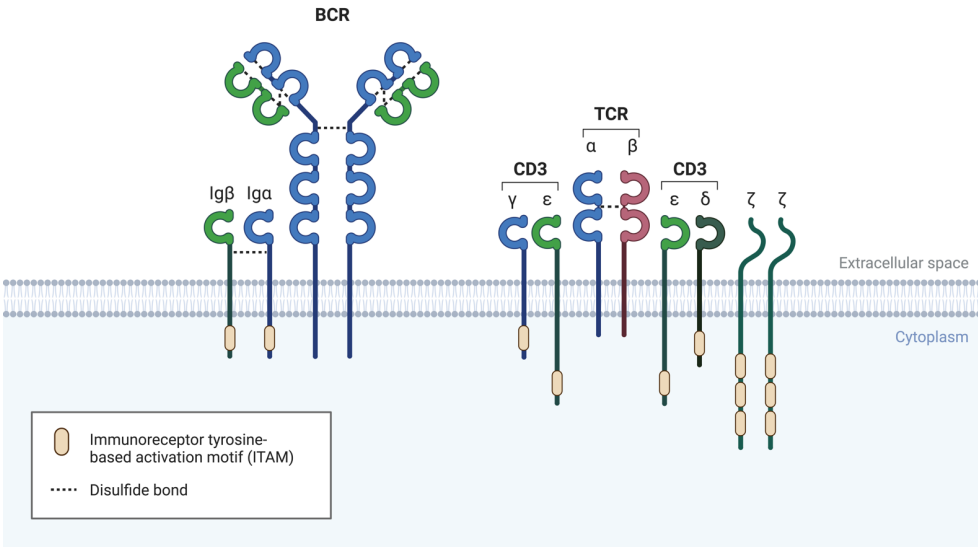


Figure 3. B cell receptor (BCR) and T cell receptor (TCR) structure. Reprinted from by BioRender.com (2022).

The initiation of the B cell derived humoral immune response requires that antigen comes in contact with the BCR. This occurs primarily in the peripheral lymphoid tissues. Here, B cells encounter complement-coated (opsonized) antigens displayed on follicular dendritic cells (FDC) which triggers BCR and complement receptor 2 (CR2/CD21) signals. This stimulation results in upregulation of surface molecules, antigen internalization and processing. Depending on the strength of the signal (level of engagement of co-receptors and number of BCRs) the B cell response will be either T cell independent or dependent. A strong signal, such as induced by bacterial lipopolysaccharides will promote T cell independent routes, and protein-based weaker signals drive T cell dependent proliferation. If the antigen is peptide-containing it will be displayed with MHC class II to CD4 Tfh cells in the context of costimulatory signals (CD40/CD40L and ICOSL/ICOS interactions) and cytokines[41] (Figure 4). The activation of B cells with T cell help will result in a long-lasting response, class-switched, high affinity antibodies and immunologic memory, in contrast to the T cell independent route.

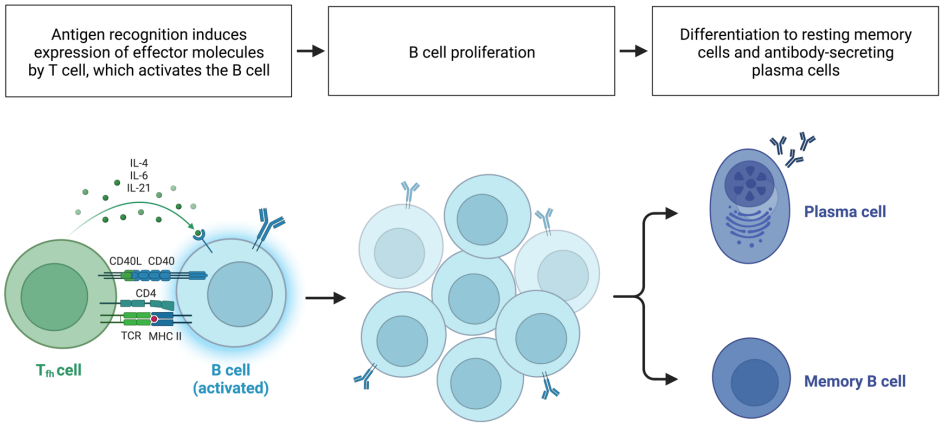


Figure 4. Steps in B cell differentiation. Reprinted from BioRender.com (2022).

An important hallmark of the humoral response is the gradual increase in antibody affinity during an infection. This is made possible by somatic hypermutation (SHM) of variable regions of immunoglobulin genes and selection of mutated B cells based on their antigen affinity, so called affinity maturation (Figure 5). These processes occur in GCs within the secondary lymphoid organs upon exposure to an antigen. The GC is divided into two compartments, light zone (LZ) and dark zone (DZ). Affinity maturation is executed in cycles of proliferation and SHM in the DZ, followed by antigen-driven, affinity-dependent selection in the LZ. The result is plasma cells and memory cells with progressive increase in antibody affinity during the primary response and upon re-infection (or immunization) of the same antigen[42]. Short-lived plasma cells function and reside in the tissue where they are formed (e.g. lymph nodes and spleen), producing large amounts of antibodies over a limited time (days) and then undergo apoptosis (days). Most long-lived plasma cells migrate to the bone marrow where they continue to produce and secrete low levels of antibodies (for decades)[43, 44]. A large number of human memory B cells has been defined[35] and can be categorized into GC dependent or independent memory B cells[45].

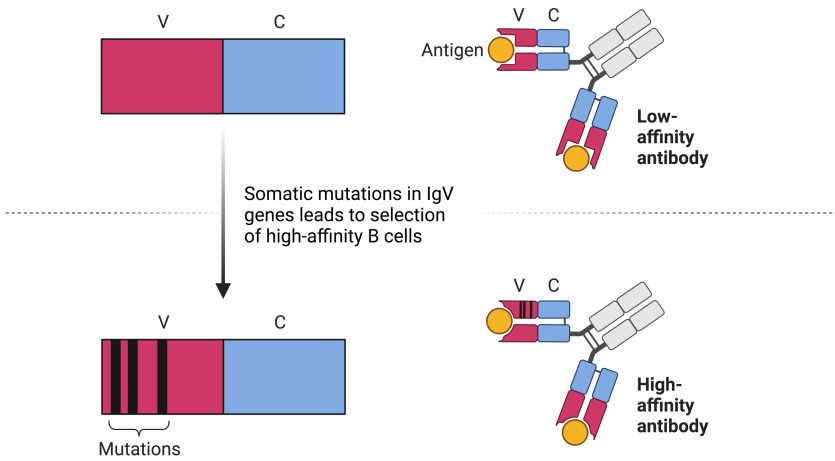


Figure 5. Somatic hypermutation allows for generation of higher affinity BCRs. Reprinted from BioRender.com (2022).

Cytokine signals and/or other extracellular influences transmitted to B cells during proliferation and maturation will determine if the produced immunoglobulin will be anchored in the cell membrane or secreted. These signals will also activate immunoglobulin class switching (class-switch recombination, CSR), leading to immunoglobulins with the same specificity but a different constant region[46]. By properties built into their structure, soluble secreted antibodies can execute several important functions. The fragment antigen binding (Fab) region of the antibody recognizes a specific antigen, and the fragment crystallizable (Fc) region recruits effector immune cells and can activate complement. There are several isotypes of secreted antibodies: IgM is the initial antibody produced in the immune response. Its pentameric structure allows ten antigen binding sites, but relatively low specific affinity, resulting in efficient binding of microorganisms. Due to the risk of formation of large immune complexes, its production is downregulated with increasing levels of IgG. Antibody-dependent cellular cytotoxicity (ADCC) is a lytic mechanism, similar to that of CD8 T cells, mediated by effector cells, e.g. NK cells, that carries receptors for the Fc portion of IgG. Interaction between Fc and Fc gamma receptors (FcγRs) connects the opsonized target cell and the immune effector cell. The specificity of the killing is determined by the specificity of the antibody and not the effector cells[47, 48]. The FcγRs are transmembrane receptors on primarily innate, but also some adaptive immune cells. The Fc portion of IgG and IgM can also bind the complement protein C1q, triggering complement-dependent cytotoxicity (CDC), resulting in formation of a membrane attack complex (MAC) and target cell lysis. IgG can be divided into four subclasses, IgG1, IgG2, IgG3, and IgG4, with different biological properties. Microbial protein antigens mainly evoke

IgG1 and IgG3 responses, whereas microbial polysaccharide antigens predominantly trigger an IgG2 response. The complement system is strongly activated by IgG1, IgG3 and IgM, weakly by IgG2 and not by IgG4. IgA is the major antibody of secretions, with capacity to form dimers, and has two subclasses, IgA1 and IgA2. The main property of IgE is to bind to mast cells and promote their degranulation during allergic reactions and parasitic infections. IgD is primarily found as a receptor on the surface of mature B cells, where it may exert a regulatory function.

Immunological tolerance

The immune system evolved to recognize and efficiently combat foreign pathogens and endogenous transformed cells, and at the same time avoid harmful reactions towards self-tissues, so called immune tolerance[49]. Efficient mechanisms for tolerance are crucial for the adaptive immune system where the randomized somatic recombination of T and B cell receptors generate cell clones with high affinity towards self-tissues. A loss of tolerance will lead to autoimmune disorders[50]. The mechanisms generating immunological tolerance can be categorized into central or peripheral.

Central T cell tolerance

A key feature of the TCR maturation process in the thymus is the ability to bind the combination of antigen associated with self-peptides of the MHC molecules, so called positive selection[51]. Subsequently, T cells with too strong binding affinity to self-peptides (autoreactive) are removed. This process, termed negative selection, is conveyed by medullary thymic epithelial cells (mTECs) and bone marrow-derived dendritic cells[52]. The mTECs express a transcriptional activator called the autoimmune regulator (AIRE), leading to exposure of developing T cells to peptides derived from self-proteins. This results in efficient elimination of autoreactive CD4 and CD8 T cells during the maturation process. Mutations in AIRE can cause autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED)[53], confirming its role in tolerance induction.

Peripheral T cell tolerance

Many self-reactive T cells escape the negative selection process of the central tolerance mechanism. Therefore, additional peripheral mechanisms are in place to maintain self-tolerance. These are conveyed by different cell types of the adaptive and innate immune system including APCs and adaptive signalling of the T cells themselves. As previously mentioned, T cell signalling requires costimulatory signals via e.g. the CD28 receptor in combination with TCR-stimulation to trigger proliferation, differentiation, and survival. Blockade of costimulation upon TCR-activation leads to apoptotic cell death, inactivation of the antigen specific clone and

tolerance[54]. In addition to costimulatory pathways, there are negative regulators of T cell activation, so called check points. The T cell surface receptors CTLA-4 and programmed cell death protein 1 (PD-1) can induce tolerance when activated by their ligands. Activation or inhibition of these pathways have had a significant impact on cancer treatment[55], autoimmune disease, and immunosuppression in organ transplantation. Autoreactive immune responses can also be targeted by specialized T cell types in the periphery such as Treg cells, type 1 regulatory T (Tr1) cells and type 3 helper T (Th3) cells[56]. tTregs, which display high self-reactivity, are formed during negative selection in the thymus under the influence of a genetic master transcriptional repressor, forkhead box P3 (FoxP3). Mutations in the FoxP3 gene can cause the autoimmune IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked)[57]. Peripheral T cells with low self-reactivity can develop into pTregs (peripheral Treg). Tregs in the periphery counter autoreactivity and promote tissue repair[58]. In addition, APCs (including certain B cells) can via inhibition of costimulation and cytokine production, interact with Tregs to control tolerance, by affecting differentiation, trafficking and function[59].

Central B cell tolerance

For B cells, central tolerance refers to regulation of autoreactivity during maturation in the bone marrow. Peripheral tolerance takes place at later development stages in secondary lymphoid organs. Of the two, central tolerance is the most important to decrease the amount of autoreactive B cells[60]. In the bone marrow, strong, multivalent, antigen stimulation (over-stimulation) of the pre-B cell BCR can induce apoptosis of self-reactive cells[61]. Further, the immature B cell can respond to receptor over-stimulation by upregulating genetic recombination and altering the affinity of the BCR to self-antigens (receptor editing). Receptor editing and clonal deletion reduces autoreactivity from 50–75% in the bone marrow to 20-40% in the transitional/immature and naive compartments[62].

Peripheral B cell tolerance

A large amount of autoreactive B cells is present outside the bone marrow, escaping central tolerance mechanisms. Further, B cells can become autoreactive through GC-induced SHM. Functional inactivation (anergy) is one of the most important mechanisms controlling B cell tolerance. In secondary lymphoid organs, B cells can become anergic when they are stimulated with antigen without T cell help. Anergic B cells do not respond to BCR stimulation and are in a state of cellular arrest. These cells quickly die off unless the antigen signal is removed. BCR desensitization involves genetic modification of autoreactive BCRs in the form of light chain recombination, or downregulation of such receptors. Similar to central tolerance, clonal deletion of autoreactive B cells can occur in the T cell zones of the spleen or lymph nodes. In addition, CD22 and Siglec-G are receptors of the Siglec family that inhibit the BCR signal and can dampen B cell autoreactivity[50, 62-64]. The term regulatory B cell (Breg) has been defined as all B cells that supress immune

responses. In addition to limiting ongoing immune activity, Bregs participate in the maintenance of tolerance. Bregs comprise about 0.5% of the total population of B cells in humans. Initially, IL-10 was proposed as the defining interleukin, but other molecules have since been associated with Bregs such as e.g. IL-35 and TGF-β, as well as the cell surface proteins CD1d and PD-1L[65-67].

Autoimmune disease

Autoimmune diseases are derived from genetic and environmental factors, characterized by loss of tolerance to self-tissues leading to chronic inflammation and local tissue destruction. The innate and adaptive immune systems are both proposed to be intimately involved in the pathogenesis where the presence of autoreactive T and B cells is an important hallmark[68]. Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and rheumatoid arthritis are considered autoimmune diseases.

ANCA-associated vasculitis

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a group of rare autoimmune disorders characterized by necrotizing inflammation of predominantly small blood vessels and the presence of circulating ANCA[69]. Clinical disease phenotypes include granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA)[70]. ANCAs are typically IgG autoantibodies directed against constituents of azurophilic neutrophil granules and monocyte lysosomes, primarily proteinase 3 (PR3) and myeloperoxidase (MPO)[69]. PR3-ANCA is associated with GPA (75%), whereas MPO-ANCA is more commonly associated with MPA (60%). ANCAs are present in approximately 50% of patients with EGPA, typically MPO-ANCA[69, 71]. As the main clinical phenotypes of AAV overlap considerably it has been proposed that the specific targets of ANCA might provide more homogeneous groups of patients than clinical diagnosis. This is of importance because ANCA specificity affects the disease progression, initial response to therapy, risk of disease relapse and long-term prognosis[72, 73].

The estimated prevalence of AAV is somewhat uncertain but ranges between 30–218 per million around the world[74]. The incidence of AAV is about 20 per million per year in Europe and North America, with a slight male preponderance. Peak incidence occurs in the 60 to 70-year age range[69].

Constitutional symptoms are prominent in AAV and the majority of patients have renal involvement in terms of rapidly progressing glomerulonephritis. Extrarenal manifestations of AAV may be upper and lower respiratory tract involvement,

hearing loss, scleritis/uveitis, cutaneous lesions and rash, peripheral neuropathy, mesenteric vasculitis and cardiovascular involvement[69]. Birmingham Vasculitis Activity Score version 3 (BVAS3) can be used to estimate disease activity in AAV[75].

The pathogenesis is multifactorial and influenced by genetics, environmental factors and responses of the innate and adaptive immune system[76]. Center stage is loss of T and B cell tolerance to PR3 or MPO. Autoantigen-specific T cells become activated and differentiate into T helper cells, including Tfh cells that interact with B cells, Th1 and Th17 cells[77]. As precursors of antibody-secreting plasma cells, B cells have a central role in the pathogenesis of AAV[78]. In addition, B cells can act as antigen presenting cells and hence initiate T cell responses by providing costimulatory signals and secrete cytokines and growth factors[79]. B cells also regulate immunological functions by suppressing T cell proliferation and producing proinflammatory cytokines, such as IFN-γ, TNF-α, and IL-17[80]. Further, the efficacy of B cell depletion therapy in AAV, e.g. rituximab, a monoclonal antibody against CD20, supports the importance of B cells in the pathogenesis.

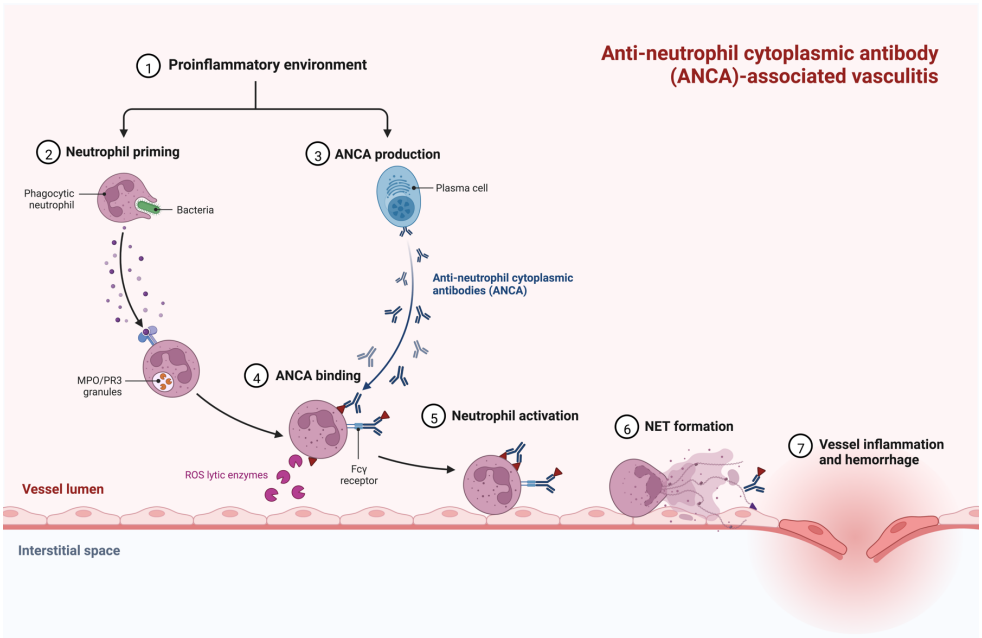


Figure 6. Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis. A proinflammatory environment (1) initiates the production of ANCA by plasma cells (2) as well as priming of neutrophils through cytokines (3). ANCA bind to myeloperoxidase (MPO) and proteinase 3 (PR3) on the surface of primed neutrophils (4) causing the release of reactive oxygen species (ROS) and lytic enzymes and excessive activation (5). Subsequent release of neutrophil extracellular traps (NETs) (6) causes the development of ANCA vasculitis with vessel inflammation and hemorrhages (7). Adapted from BioRender.com (2022).

ANCAs have been proposed to cause vasculitis by activating primed neutrophils (and monocytes) resulting in proinflammatory responses, including production and release of ROS, lytic enzymes, matrix metalloproteinases, and NETs, which can damage small blood vessels[72, 81] (Figure 6).

As the glomerulonephritis seen in AAV displays little immunoglobulin and complement deposition in the capillary walls, the complement system was initially thought to be unrelated to the pathogenesis[82, 83]. However, the alternative pathway has emerged to be important for the development of the leukocytoclastic inflammation seen in acute AAV[6]. Further, antagonism of the complement peptide C5a, a powerful chemoattractant for neutrophils, ameliorated necrotizing glomerulonephritis in an animal model of AAV[84].

Treatment of AAV consists of induction of remission followed by maintenance treatment to prevent disease relapse. The aim of modern treatment regimens is to limit treatment toxicity by decreasing cumulative exposure to cyclophosphamide and glucocorticoids, increasing the use of rituximab, and introducing therapies with less toxicity e.g. methotrexate and mycophenolate mofetil[85, 86]. There is a number of emerging treatments for AAV directed towards the complement system (e.g. C5a receptor inhibitor), the autoantigen MPO, intracellular pathways triggered by ANCA, as well as strategies aiming to prevent the formation of NETs[87, 88]. Historically, the prognosis of AAV was poor, with a mean survival of 5 months for patients with GPA[89]. Modern treatment regimens have decreased the mortality rates, and the estimated 5-year survival is 74-91% and 45-76% for GPA and MPA, respectively[90].

Rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the synovium and erosions in peripheral joints[91], and may involve extra-articular organs[92]. RA can lead to severe disability and reduced life span[93]. Several types of immune cells including B cells, T cells and macrophages have been suggested to contribute to the inflammation in RA. B cells activate T cells and secrete autoantibodies, such as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), and proinflammatory cytokines. T cells promote inflammation by activating fibroblasts, and macrophages which release cytokines[94].

The clinical presentation is highly variable and some individuals are negative for autoantibodies (seronegative RA). Classification criteria for RA were updated by American College of Rheumatology and European League Against Rheumatism in 2010[95]. The categories of the criteria rely on scores for joint symptoms, serology (including RF and/or ACPA), symptom duration and acute-phase reactants (C-reactive protein (CRP) and/or erythrocyte sedimentation rate (ESR)). Disease

activity score of 28 examined joints, DAS28, can be used to assess disease activity in RA[96].

The etiology is complex and involves a combination of environmental factors and genetic susceptibility[97]. More than 150 candidate loci with polymorphisms associate with RA, and several alleles of HLA-DRB1 that code a shared epitope (a five amino acid sequence) are associated with high risk for autoantibody-positive RA[98, 99]. Further, smoking is one of the most established risk factors of RA[100].

The prevalence of RA has recently been estimated in a meta-analysis and reached 56 per 10.000 around the world[101], and is higher than previous estimates[102]. RA is more common in females. The published incidence levels of RA vary but was 4.1 per 10.000 in a US study[103].

Patients with RA, and other autoimmune diseases, exhibit an increased risk of infections. The cause is multifactorial and likely a combination of the autoimmune nature of the disease and current pharmacological treatment such as glucocorticoids and different types of disease-modifying antirheumatic drugs (DMARDs)[104, 105]. Therefore, immunization against vaccine-preventable diseases is important in RA[105].

Treatment of RA consists of agents to relieve pain and decrease inflammation, including NSAID and corticosteroids, in combination with strategies to promote remission by slowing or stopping the progression of joint destruction, including DMARDs[106]. Methotrexate (MTX) is the most commonly used DMARD, and first-line treatment in RA. MTX was initially developed in the oncology field and is a folate antagonist inhibiting DNA and RNA synthesis. However, low dose MTX suppresses inflammation in RA by regulating many proinflammatory cell lineages, via mechanisms involving adenosine signalling, inhibition of nuclear factor-κB (NF-κB), inhibition of dihydrofolate reductase-related transmethylation reactions, and generation of ROS[107]. Other synthetic DMARDs include hydroxychloroquine, sulfasalazine and Janus kinase (JAK) inhibitors. Biological DMARDs bring rapid symptom relief and include B cell depletion and inhibition of TNF-α, IL-1, IL-6 and T cell activation[106, 108].

Vaccine immunology

Edward Jenner's contribution to immunization and the eradication of smallpox is regarded as the foundation of immunology. However, he was not the first to suggest that cowpox infection could result in immunity to smallpox nor the first to attempt inoculation of cowpox[109]. Several centuries earlier attempts were made to induce immunity to smallpox using dried crusts from smallpox lesions[110].

Immunization can be passive or active, and both of these can be either natural or artificial. Passive immunization occurs when antibodies are transferred to an individual, generating a temporary immunity by neutralizing the pathogenicity of a specific microorganism or toxin. An example of natural passive immunization is the passage of maternal antibodies through the placenta to the fetus. Artificial passive immunization is e.g. administration of human immune (gamma) globulin. Unlike passive immunization, active immunization stimulates the host immune system to develop long-term immunity. Natural active immunization can be exemplified by societal exposure to common influenza virus. Artificial active immunization occurs following vaccine administration[110].

Both innate and adaptive immune responses are necessary for effective immunization, whether to an actual invading pathogen or to a vaccine. Ideally, a vaccine triggers both the humoral and cell-mediated parts of the adaptive immune system, producing effector cells to deal with the current antigen, and memory cells ready for future exposure to the same antigen.

Immune response to pneumococcal conjugate vaccine

Autoimmune inflammatory rheumatic diseases are accompanied by an increased risk of infections. Therefore, immunization is important in these diseases[105].

Streptococcus pneumoniae causes both mild infections and severe disease such as pneumonia and meningitis, and serotypes with high infectivity have been selected for vaccine development. Current vaccines consist of unconjugated purified polysaccharides (PPSV), which activate B cells without stimulating memory B cell production, and protein-conjugated formulations (PCV), which lead to more robust and prolonged immune response due to T cell activation (see below)[111]. For certain patient groups, the two vaccines are given in sequence (PCV first) to augment the immune response[112].

Following the injection of the conjugated vaccine (including its aluminium phosphate adjuvant), APCs of the innate immune system, such as DC and macrophages, will recognize, internalize and process the polysaccharide-protein conjugate to be presented with MHC class II, and migrate to the draining lymph node. Here, they will activate T cells via antigen presentation to the TCR, promoting formation of GCs. In the GC, polysaccharide-specific B cells extract and internalize vaccine particles (consisting of polysaccharides and the protein carrier covalently bound) from follicular dendritic cells (FDCs) and present them to Tfh cells. Activated Tfh cells provides help to the B cell by direct cell–cell interaction and by secreting cytokines driving SHM via a process called affinity maturation. This process leads to maturation of the B cell population into plasma cells secreting IgG antibodies with high affinity to the vaccine antigen, and into switched memory B cells[113].

B cells can also recognize antigens that have not been processed by an APC and mount a T cell-independent immune response. The pneumococcal polysaccharide moiety in 13-valent pneumococcal conjugate vaccine (PCV13) will directly cross-link the BCR, activate B cells to produce IgM and promote trafficking of the B cells towards the T cell zone of secondary lymphoid organs.

Evaluating immune response to pneumococcal conjugate vaccine

Immune responses following pneumococcal vaccination can be evaluated by quantification of serotype-specific IgG, functionality of antibodies and T cell responses.

Quantifying antibody levels after PCV administration

A common method to determine antibody concentration is the enzyme-linked immunosorbent assay (ELISA) where the antigen is immobilized on a solid surface (microplate) and then the serotype-specific IgG is complexed with an antibody linked to a reporter enzyme. It is specific, sensitive and well suited for testing many samples for a specific antibody. However, monitoring of the antibody response to currently available pneumococcal vaccines require detection of many different serotype-specific IgGs in the same sample. Microsphere-based flow cytometric assays permit simultaneous detection of many antibodies from a single sample which allows high sample throughput and use of very small sample volumes[114].

In paper III, pneumococcal serotype-specific IgG concentrations were measured for 11 capsular serotypes included in PCV13 (1, 3, 4, 5, 6B, 7F, 9V, 18C, 19A, 19F and 23F), using an in-house multiplex fluorescent microsphere immunoassay (MFMI, Luminex) based on the procedure described by Lal et al.[114], with some modifications.

Differences in interlaboratory variability of current methodologies can make it challenging to set precise criteria for defining an antibody response. There is some controversy about what constitutes a meaningful serotype-specific response following immunization. It has been suggested that the overall pattern in the antibody response is the most important factor. Therefore, adequate antibody response is usually defined by the percentage of serotypes showing a predefined fold-change relative to baseline, or by an absolute concentration which has been established to be protective against disease[115, 116]. In paper III, antibody response ratio (ARR, i.e. the ratio of post- to prevaccination serotype specific IgG concentration) was calculated[117], and positive antibody response was defined as ARR ≥2 in >50% of serotypes (at least 6 of 11 serotypes)[118].

Opsonophagocytic activity against Streptococcus pneumoniae

Opsonophagocytosis by antibodies and complement is a key mechanism for clearing Streptococcus pneumoniae from the host[119]. Therefore, in vitro opsonophagocytic activity (OPA) of serotype-specific antibodies is believed to correlate to their functional activity in vivo. OPA can be measured by several techniques e.g. the viable cell-assay, flow-cytometric assays and assays utilizing radiolabelled bacteria[120]. There is generally good correlation between the concentrations of specific IgG antibodies measured by ELISA and OPA assays[121, 122]. However, discrepancies have been found for certain serotypes, low antibody concentrations and as well for specific patient groups and immunosuppressive treatments. For example, in RA patients and elderly with B cell malignancies, OPAs are considered the best functional correlate of protection, i.e. the antibody functionality rather than antibody quantity is important[123-125].

T cell response following PCV immunization

As previously detailed, T cells proliferate and differentiate to participate in the clearing of an infectious agent. Following the resolution of the infection the majority of adaptive cells, including T cells, die and leave behind memory cell subsets with different phenotypes and functions[126]. The memory T cells primed for subsequent activation via antigenic and costimulatory receptors, display an increased proliferative potential, and are much more rapid in their effector response as compared to their naive counterparts[127]. Importantly, memory T cells are very long-lived, where some subsets can survive for decades[128]. It has been shown that changes within the CD4 T cell population at older age can compromise specific responses to Streptococcus pneumoniae[129]. Different methods can be employed to assess the T cell response following PCV vaccination, e.g. determination of CD4 T cell proliferation, CD4 T cell subsets and production and release of cytokines/effector molecules[129-131].

Aims

The overall aim of this thesis was to study phenotypic alterations of peripheral immune cells in autoimmune disease using flow cytometry. The specific aims were as follows:

Paper I	To search for genetic sequence variants in the general population influencing immunoglobulin (Ig) levels using a genome-wide association study of nine Ig traits, three individual (IgA, IgG, IgM) and six composite Ig traits
Paper II	To study frequencies of B cells and subsets in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) patients, and to explore if activated B cells/subsets and immunoglobulin levels correlate with disease activity and/or tendency to relapse
Paper III	To investigate the potential role of the innate immune system in the faltering antibody response following pneumococcal vaccination in rheumatoid arthritis patients treated with methotrexate
Paper IV	To explore the relation between granulocyte/monocyte subsets and disease activity and tendency to relapse in AAV patients

Methods

Concise accounts for two of the main methods used in this thesis are given below. For full description of the methods used, see the enclosed papers.

Genome-wide association study

Many common conditions and traits, including autoimmune disease, are influenced by a combination of genetic predisposition and environmental or lifestyle factors[132]. These complex disorders are polygenic, affected by a very large number of sequence variants, where each has a limited effect on the disease or trait[133]. Early efforts to characterize genome-wide variation have demonstrated that the genome is constructed in haplotype blocks, containing sets of linked sequence variants[134]. This understanding opened up for genome-wide association studies (GWAS). Genome-wide data (from e.g. single nucleotide polymorphism arrays or whole genome sequencing) is collected to map the common variants in a large cohort, with and without a common trait (e.g. a disease). To cover the extent of the human genome, GWAS must obtain and test association for several hundred thousand independent linkage groups for polymorphisms. The results of associations with a particular trait generate a list of GWAS linkage regions in the genome to that particular trait, together with effect sizes with their directions, and probability estimates (p-values) of how likely a variant is to be associated with that trait. Importantly, the analysis does not generally identify variants with direct causal relationship with the trait or disease, but identifies an associated genetic region containing the causal variant(s). Additional studies are most often required to identify the disease-causal variants, the genes they regulate and the affected cell types driving the phenotype[135]. The growing availability of whole-genome sequence data and imputation strategies has extended the discovery to low-frequency and rare alleles, previously inaccessible to GWAS[136].

Flow cytometry

General principles

Flow cytometry has proven to be a powerful technology that simultaneously measures light scattering and fluorescence characteristics of single particles, usually cells, as they flow in a fluid stream through a beam of light (laser). The main domains of light scatter are forward scatter (FSC) which is proportional to the diameter of the particle, and side scatter (SSC, detected at 90° angle from the light path), which is related to cell complexity of internal structures (i.e. granularity). Fluorescence probes emit light proportional to the amount of probe bound to the cell or cellular component[137]. The combination of light scattering and fluorescence allows for detection of multiple parameters of individual cells, and detailed phenotyping of many subsets of cells in a heterogeneous population, such as blood. Further, by using a technique called fluorescent activated cell sorting (FACS), cells can be sorted and collected for further study.

A variety of fluorescent reagents are utilized in flow cytometry. Samples can be stained with fluorescent dyes, transfected to express fluorescent proteins, or labelled with antibodies conjugated to small organic molecules, also called fluorochromes (or fluorophores). The development of tandem dyes has expanded the number of fluorophores that are suitable for flow cytometry, by increasing the number of colours that can be detected in an experiment. A tandem fluorophore consists of two covalently attached molecules (a donor and an acceptor) that behave as a unique fluorophore with the excitation properties of the donor and the emission properties of the acceptor. This process is called fluorescence resonance energy transfer (FRET)[138]. The emission spectra of multiple fluorophores used in an experiment will partially overlap (spectral overlap), that can be corrected for by a process called compensation. Compensation is straight-forward in two or three colour flow cytometry but needs advanced software algorithms in multi-channel experiments.

Fluidics, optics, and electronics are the main components of a flow cytometer. The fluidics system delivers and focuses the sample to the laser intercept. This allows controlled and uniform illumination of a particle or cell, called hydrodynamic focusing. The optical system consists of excitation optics (lasers) that generate the visible and fluorescent light signals, and the collection optics (photomultiplier tubes and photodiodes) that detect and collect the emitted light signals. The electronic system transfers and converts the signals from the detectors into digital signals that are processed by the instrument computer[139].

Applications of flow cytometry

A common use of flow cytometry is immunophenotyping. Here, cells are stained by a cocktail of fluorochrome-conjugated antibodies targeting cellular surface antigens. A standardized nomenclature, cluster of differentiation (CD), for specific surface

antigens and the antibodies that target them has been established by the "Human Leukocyte Differentiation Antigens Workshops"[140]. Routine clinical phenotyping of blood samples can easily be achieved with eight to ten antibodies in a single tube. Blood and bone marrow are most commonly analysed but almost any cell suspension or body fluid can be stained and analysed.

In addition to surface markers, flow cytometry can be used to detect e.g. cytoplasmic and nuclear antigens, organelles, nuclei, DNA, RNA, chromosomes, cytokines, and hormones. Complex applications such as e.g. detailed investigation of cell proliferation and cell cycle have been developed for apoptosis and cancer research[141].

Methods used to analyse and interpret data

Conventional flow cytometry analysis starts with displaying the data in a dot plot followed by drawing a boundary (gate) around the dots (cells) of interest. This allows specific groups of cells to be isolated and selected for analysis or sorting for further analysis of other markers (Figure 7).

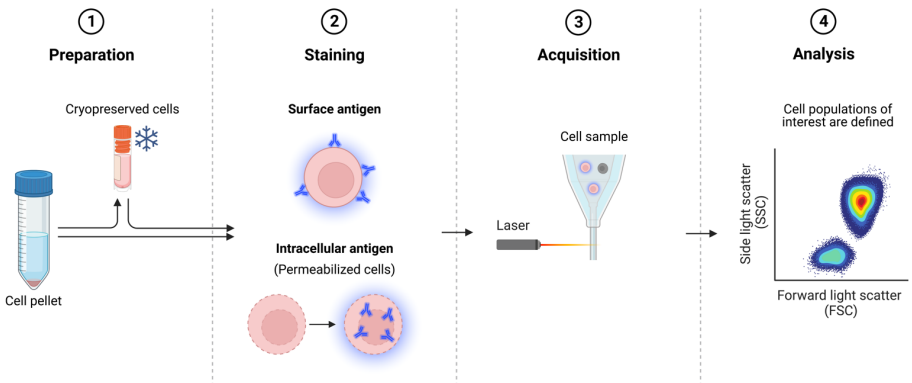


Figure 7. Flow cytometry experiment. Sample preparation (1) from blood often involves separation of mononuclear cells, and sometimes cryopreservation, before staining (2) with fluorescent antibody conjugates. Data acquisition (3) involves passing the stained cells through a laser beam and recording the fluorescence emission from all of the bound antibody conjugates. This is followed by data analysis (4), in which cell populations of interest are defined and reported on. Adapted from "Flow Cytometry Experiment", by BioRender.com (2022).

Preanalytical and analytical issues

There are a number of common flow cytometry preanalytical and analytical issues that could lead to incorrect interpretation of results. The quality of the samples used for flow cytometry is an important issue[142]. For example, clotted or hemolysed blood samples may not generate representative data. In addition, under certain conditions red blood cells might be difficult to lyse, which could affect the analysis[143].

When aiming to detect surface immunoglobulins, samples should be carefully washed to remove cytophilic antibodies. Inadequate washing might cause unusual or unexpected staining patterns.

A common strategy to get a rough estimate of viability of the sample and identify debris is to plot forward scatter (FSC) versus side scatter (SSC). Other methods include a viability dye in the analysis.

Breakdown of the antibody-conjugated dye is also a concern, especially for tandem dyes. The antibody dyes should be protected against direct light and stored according to the manufacturer's instructions to limit breakdown.

Many samples contain cell doublets which are single events that actually consist of two independent particles. The frequency of doublets could increase if the sample comes from patients with certain diseases such as B cell malignancies, is derived from incompletely disaggregated tissue samples, or could be due to high flow rates. Two different ways to separate single events from multiple particles using FSC or SSC are plotting of width vs. area or plotting area vs. height.

To be able to analyse samples with very few cells such as cerebrospinal fluid it is important that the instrument is clean and that there is no carryover from previous samples[144].

Standardization

There is a large variety of analysers, reagents, applications and protocols employed for flow cytometry to monitor immune cells of humans. Many of these variables need to be standardized for correct acquisition, analysis and comparable interpretation of the generated data. In 2012, Maecker et al.[145] outlined the current state of standardization of flow cytometry assays for immunophenotyping of peripheral blood mononuclear cells. The suggested B cell phenotyping is used in the attached Paper I-II.

The flow cytometry technology was used in all four papers included in this thesis. For details about the monoclonal fluorescent-labelled antibodies used for immunophenotyping and gating strategies, see enclosed papers (B cell immunophenotyping Paper I-II, monocyte and granulocyte immunophenotyping Paper III-IV). The targeted cell surface molecules, their cellular expression and their most renowned functions are presented in the Table 1.

Table 1. Cell surface markers used for immunophenotyping in Paper I-IV

Surface marker	Cellular expression	Functions
CD10	B and T cell precursors, fibroblasts, mature neutrophils, bone marrow stromal cells	Endopeptidase, involved in B cell development
CD11b	Granulocytes, monocytes, NK cells, subsets of T and B cells, DC	Cell adhesion, apoptosis, chemotaxis, neutrophil activation
CD14	Monocytes, macrophages, Langerhans cells, granulocytes	Receptor of lipopolysaccharide (LPS), involved in clearance of gram-negative pathogens, in upregulation of adhesion molecules and cytokines
CD16	Neutrophils, NK cells, macrophages, monocytes	Low affinity IgG receptor III (FcγRIII), mediates NK cell activation, phagocytosis and antibody-dependent cellular cytotoxicity (ADCC)
CD19	B cells (not plasma cells), follicular DC	Involved in B cell development, activation and differentiation. Forms a complex with CD21 and CD81, functions as a BCR co-receptor
CD24	B cells (not plasma cells), granulocytes, epithelial cells, monocytes, follicular DC	Regulation of B cell proliferation and differentiation
CD27	T cells, medullary thymocytes, B cell subset, NK cells	Costimulation of T cell activation, regulation of B cell differentiation and proliferation
CD38	Variable on most hematopoietic cells and some non-hematopoietic cells. High on plasma cells, early T and B cells, activated T and B cells	Regulates cell activation, proliferation, adhesion
CD45	Hematopoietic cells (except erythrocytes and thrombocytes)	Regulates cell growth, differentiation, cell cycle, oncogenic transformation. Critical for T and B cell receptor-mediated activation
CD62L	Most peripheral B cells, subsets of T and NK cells, monocytes, granulocytes	(L-selectin) Leukocyte rolling and homing
CD69	Inducible on activated leukocytes incl. T cells, immature thymocytes, B cells, NK cells, monocytes, neutrophils, eosinophils. Expressed by mature thymocytes, platelets	Involved in lymphocyte, monocyte, platelet activation. Functional role in redirected lysis mediated by activated NK cells
CD80	Activated B and T cells, macrophages, DC, monocytes	Costimulation (with CD28) of T cell activation through the CD3 complex. Costimulation (with CTLA-4) of an inhibitory signal for T cell activation
CD95	T and B lymphocytes, monocytes, neutrophils, fibroblasts	Upregulated by activation. CD178 (Fas ligand) binding to CD95 (Fas) induces apoptosis. Role in maintenance of peripheral tolerance
CD177	Granulocytes, bone marrow progenitors (early erythroblasts, megakaryocytes)	(Neutrophil specific antigen 1) Involved in allogeneic and autoimmune responses to neutrophils
CD193	High in eosinophils, basophils. Detected in Th1 and Th2 cells, airway epithelial cells	(CCR3) Involved in allergic diseases (e.g. bronchial asthma and allergic rhinitis), entry co-receptor for HIV-1
HLA-DR	B cells, activated T cells, monocytes, macrophages, DC, non-professional APCs	In conjunction with the CD3/TCR complex and CD4 molecules, critical for efficient peptide presentation to $CD4^+$ T cells
IgA	IgA^+ cells were gated among switched memory B cells (Paper I)	
IgD	Naive B cells (expression lost after Ig isotype switch)	After antigen binding, IgD signals through CD79a/CD79b, resulting in activation of B cell
Siglec-8	Eosinophils, mast cells, basophils (lower expression)	Inhibits release of histamine and prostaglandin D2, involved in induction of apoptosis

Adapted from https://docs.abcam.com/pdf/immunology/Guide-to-human-CD-antigens.pdf, https://www.biolegend.com

Results and discussion

Selected main findings are included and discussed below. For complete account of the results, see the enclosed papers.

Sequence variants influencing Ig levels (paper I)

Here, the genetic basis of variability in immunoglobulin (Ig) levels in the general population was explored in an extensive genome-wide association study of nine Ig traits, three individual (IgA, IgG, IgM) and six composite Ig traits (Table 2).

Table 2. Definition of individual and composite Ig traits used for association analysis

Ig trait	Definition	Phenotype
IgA	Log-standardized IgA	IgA level
IgG	Log-standardized IgG	IgG level
IgM	Log-standardized IgM	IgM level
AGM	Log-standardized IgA x IgG x IgM	total Ig production
AG	Log-standardized IgA x IgG	total class-switched Ig production
AG/M	Log-standardized IgA x IgG / IgM	ratio of class-switched to non-class-switched immunoglobulins
A/M	Log-standardized IgA / IgM	IgA-specific class switching
G/M	Log-standardized IgG / IgM	IgG-specific class switching
A/G	Log-standardized IgA / IgG	ratio of IgA to IgG production (to capture effects acting in opposite directions on the two isotypes)

Adapted from paper I, supplementary table 1.

A discovery data set of Icelandic subjects and a follow-up data set of Swedish subjects were generated. For the Icelandic data set, extensive whole-genome sequencing and single nucleotide polymorphism (SNP) microarray data were used, as well as available IgA, IgG and IgM values (>14.000-16.000 values/isotype) from clinical laboratories. Based on association results, selected genetic sequence variants were followed-up in a cohort of Swedish blood donors (~2000 subjects).

Thirty-eight of the independent sequence variants (in combined analysis of the Icelandic and Swedish data) reached significant association with one or more Ig traits (paper I, table 1). Additionally, five previously reported associations were replicated. Taken together, these 43 lead variants were accounted for by 32 genetic

loci. The associations with IgA, IgG and IgM were isotype specific. The composite traits showed considerable cross-trait association likely related to their construction (Table 2).

Identification of candidate genes and associations with human diseases

To identify possible genes underlying the immunoglobulin associations, 33 genes that overlapped extended regions defined by the variants were selected as probable candidate genes based on different criteria (details in paper I and its online methods). To these, 11 genes with immune-related functions contained in the immunoglobulin-associated loci were added bringing the number of probable candidate genes to 44. Pathway analysis showed that the defined set of candidate genes was enriched for genes involved in cellular growth and proliferation, cell-to-cell signalling as well as infectious and inflammatory diseases.

Using the GWAS catalogue[146] associations with relevant diseases were probed for the 43 lead sequence variants. Considerable overlap was found with variants related to e.g. lymphoid malignancies, the human leukocyte antigen (HLA) region and autoimmune diseases, indicating that genetic alterations affecting Ig levels may be involved in immune-related diseases. Some of the genes identified underlie heritable immunodeficiencies, sensitivity to mycobacterial infections, RIDDLE syndrome and as well hematologic malignancies.

Gene expression in human hematopoiesis and effects of sequence variants on blood cell development

Using gene expression profiles of different sets of hematopoietic cell types, expression profiles of most of the candidate genes were investigated. The candidate genes displayed their highest expression in lymphoid cell types with enrichment in the B cell lineage, supporting the relevance of identified candidate genes to immunoglobulin biology.

One mechanism by which sequence variants could influence Ig levels is by interfering with development of blood cells, particularly B lymphocytes. To probe this, frequencies of eight B cell subsets in 2,207 genotyped Swedish blood donors were determined with flow cytometry. Two variants showed significant association: an A/M variant upstream of the gene for HLA-B associated with increased frequency of switch memory B cells, and an IgA variant upstream of activating signal cointegrator 1 complex subunit 2 (ASCC2), associated with lower frequency of transitional naive B cells. ASCC2 is implicated in DNA repair mechanisms. These variants (and seven more) also associated with lymphocyte or total white blood cell levels in the Icelandic cohort. If confirmed in larger studies, these results indicate that some of the identified variants that associate with Ig traits may also influence blood and immune cell frequencies.

Associations with individual immunoglobulin traits – IgA, IgG and IgM

The strongest association with IgA was represented by a rare variant in the *RUNX3* P1 promoter with a large negative effect. Runx is an evolutionary conserved family of transcription factors regulating genes involved in embryonic development and cell differentiation[147]. In mammals, three isoforms (runx1, runx2, and runx3) have been described. Runx1 is implicated in hematopoiesis and angiogenesis; runx2 in bone formation and runx3 in differentiation/homeostasis of several cell types - CD8 T cells, DCs, Langerhans cells, dorsal root ganglion neurons, and gastrointestinal epithelial cells. Runx proteins have been shown to play an essential role in IgA class switching acting downstream of retinoic acid and TGF-β1 signalling[148]. The *RUNX* genes have alternative promoters, proximal (P2) and distal (P1), and the transcripts also undergo alternative splicing, resulting in multiple isoforms.

In paper I experimental data suggest that the variant rs188468174[C>T] in the *RUNX3* P1 promoter lowers IgA levels by shifting *RUNX3* isoform proportions toward the long isoform. IgA also associated with more common sequence variants (paper I, table 1) related to e.g. metabolism of retinoic acid, autoimmune diseases, myocardial infarction, and the synthesis of proinflammatory eicosanoids.

The strongest IgG associations mapped to the Fcγ receptor locus including a rare in-frame deletion in *FCGR2B* (coding for the Fc fragment of IgG receptor IIb) with a large positive effect. The FcγRIIb receptor normally suppresses IgG production upon IgG binding which suggests loss of function caused by the in-frame deletion. Validating experiments demonstrated that in-frame deletion in *FCGR2B* abolishes IgG binding to the encoded receptor (paper I, figure 4a, b).

Nine independent associations were found with IgM. The strongest association was linked to *KLF10* (Krüppel-like factor 10) as the likely candidate gene. The Krüppel-like factor (KLF) family of transcription factors regulates many physiological systems including the hematological and immune systems and is involved in diverse disorders such as obesity, cardiovascular disease, cancer, and inflammatory conditions[149]. Of relevance to the association found with IgM, KLF10 has been proposed to potentiate the TGF-β signalling pathway by altering expression and activity of intracellular effector proteins[150]. TGF-β has been reported to inhibit release and production of immunoglobulins, including IgM[151]. Other IgM associations mapped to loci containing genes previously implicated in the regulation of IgM levels, where two of the signals correlate with reported autoimmune disease associations and two correlate with reported lymphoid malignancy associations.

Associations with composite traits

Analysis of the composite Ig traits proved informative, contributing new associations to genes in the biological processes that the constructed traits were designed to capture. Importantly, a majority of the associations to the composite Ig traits were not detected by analysis of IgA, IgG or IgM individually, showing that

these constructed traits also captured unique information. For example, a variant upstream of the gene for a proliferation-inducing ligand (APRIL, also known as tumour necrosis factor ligand superfamily member 13), a key factor for B cell response and plasma cell development, associated with the proxy for total immunoglobulin, AGM. Further, a variant related to TNF receptor-associated factor 3 (TRAF3) associated with the proxies for general class switching, AG/M and AG. TRAF3 is a negative regulator in multiple aspects of B cell biology and regulates CD40-driven class switch recombination[152, 153].

Several variants in the immunoglobulin heavy chain locus (IGH) associated with the isotype-specific class switching proxy traits A/G or A/M, possibly through direct effects on the class-switching process.

Two loci may point to novel mechanisms in immunoglobulin regulation. First, a missense mutation in the adenine DNA glycosylase gene *MUTYH* associated with A/M. MUTYH is a DNA glycosylase involved in the restoration of post-replicative mispairs in double-stranded DNA, catalysing the base excision DNA repair mechanism (BER)[154, 155]. BER is involved in immunoglobulin somatic hypermutation and class switching[156]. The results suggest that MUTYH may have a role in immunoglobulin class switching which has not previously been described. Second, a missense mutation in killer cell lectin like receptor C2 (KLRC2) associated with A/G. While KLRC2 is known to mediate recognition of HLA-E molecules by NK cells[157], it has not previously been associated with humoral immunity.

Many genes with well-established roles in class switching were absent from the composite-trait-associated loci, suggesting that the proxy traits may not capture all genetic variability in the mechanisms that can affect Ig levels.

Associations of markers within the HLA region

Eight immunoglobulin association signals clustered to four loci in the HLA region (paper I, table 1). To search for associations with classical HLA alleles that are not captured by SNPs, association of all nine Ig traits were tested with classical HLA alleles (paper I, online methods). In total, 13 HLA alleles associated with an Ig trait. Some of the signals link effects on Ig levels to known strong genetic risk factors for immunological disease. For example, an association was found between HLA-B27 and IgA. Despite its central role in predisposition to autoimmune disease such as spondyloarthropathies, the underlying mechanism is not understood. Here, HLA-B27 was connected to IgA levels, which is consistent with the theory that IgA has a major role in mucosal defence, and gut mucosal immunity is involved in the development of ankylosing spondylitis[158, 159].

In summary

The genetic basis of variability in Ig levels in the general population was explored in an extensive genome-wide association study of nine Ig traits. Thirty-eight new variants and five known variants associated with IgA, IgG or IgM levels or with composite Ig traits, accounted for by 32 genetic loci. These sequence variants explained 4.3-8.7% of the variance in the nine Ig traits. Variants at these loci have been shown to affect the risk of autoimmune diseases, blood malignancies and influence blood cell development. Notable associations include a rare variant at RUNX3 decreasing IgA levels by shifting isoform proportions, a rare in-frame deletion in FCGR2B abolishing IgG binding to the encoded receptor, four IGH locus variants influencing class switching, and ten new associations with the HLA region.

B cell frequencies in ANCA-associated vasculitis (paper II)

B cells are thought to play a central role in the pathogenesis of ANCA-associated vasculitis (AAV) and this autoimmune disease is effectively treated by B cell depletion.

Here, we studied if AAV patients have altered frequencies of B cells and subsets in peripheral blood. Using flow cytometry, the frequencies of $CD19^+$ B cells and subsets were assessed in peripheral blood from 106 patients with AAV and 134 healthy controls. In addition, serum concentrations of immunoglobulin A, G, and M were measured, and clinical data retrieved.

Specific changes in frequencies of $CD19^+$ B cells and their subsets in AAV patients

AAV patients displayed, in relation to healthy controls, a decreased frequency of B cells of lymphocytes (5.1% vs. 8.3%) and total B cell number (paper II, figure 1, and Figure 8 below).

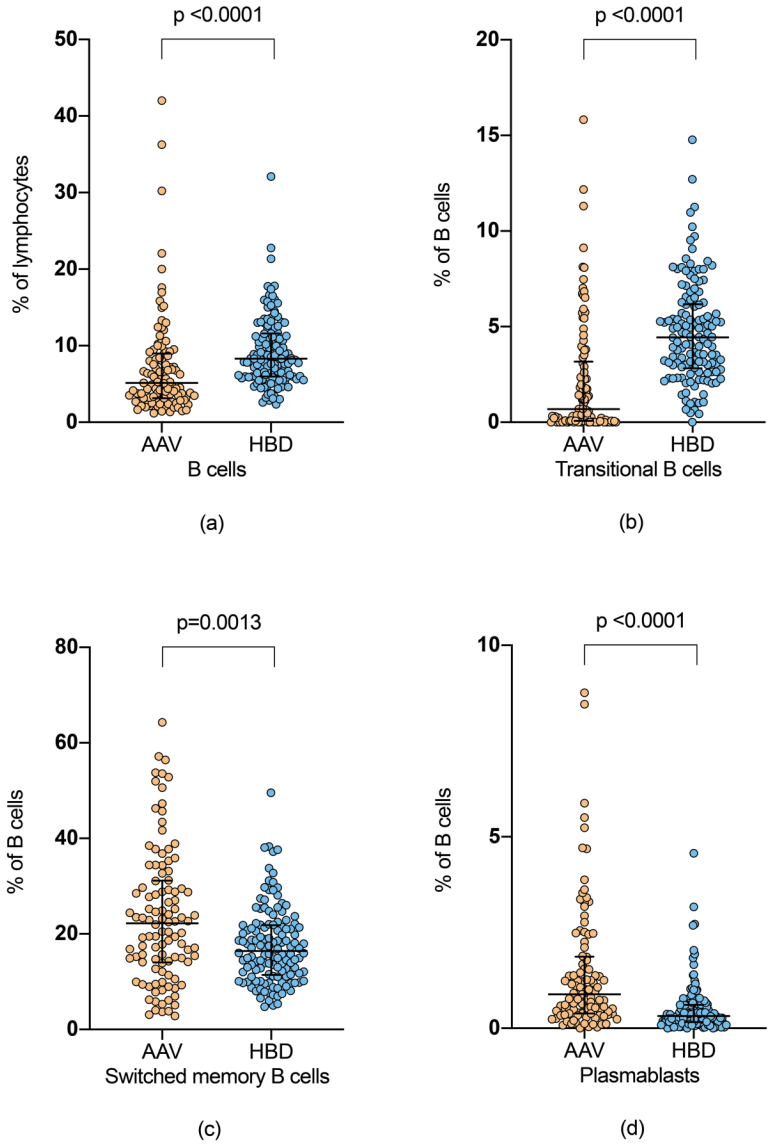


Figure 8. Comparisons of B cells and subsets between vasculitis patients and healthy blood donors. Percentage of (a) $CD19^+$ B cells of lymphocytes, (b) transitional B cells of $CD19^+$ B cells, (c) switched memory B cells of $CD19^+$ B cells, and (d) plasmablasts of $CD19^+$ B cells, in peripheral blood from patients with anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV, n=106) and healthy blood donors (HBD, n=134). Mann-Whitney *U* test was used to calculate the level of significance. Data are presented with medians and interquartile ranges. Reprinted from paper II, figure 1.

Other studies investigating GPA patients in remission have demonstrated lower absolute numbers of circulating $CD19^+$ B cells[160] and lower B cell frequency[161], but another study by Lepse et al. found no difference in B cell frequency between AAV

patients and healthy controls[162]. Transitional B cells can produce IL-10 and regulate CD4 T cell proliferation and differentiation toward T helper effector cells[163]. Here we detected a decrease in percentage of transitional B cells (0.7% vs. 4.4%) in patients (paper II, figure 1, and Figure 8 above). In a study by von Borstel et al. comparing GPA patients with future relapse, nonrelapsing patients, and healthy controls, no differences in transitional B cell frequencies were found[161]. In support of our findings, low frequencies of transitional B cells have been noted in neuroimmunological diseases, including multiple sclerosis[164] and neuromyelitis optica[165]. However, the frequency of $CD24^{high}CD38^{high}$ transitional B cells is elevated in patients with systemic lupus erythematosus (SLE) and Sjögren's syndrome[166].

Memory B cells are optimized to interact with T cells and to yield strong antibody responses. High frequencies of memory B cells are associated with poor clinical response to rituximab (anti-CD20) treatment[167]. We found an expansion of switched memory B cells (22.3% vs. 16.5%) in AAV patients (paper II, figure 1, and Figure 8 above), and patients with medication had a higher percentage compared to the nonmedication group and healthy controls. Decreased proportion of circulating $CD27^{+}$ memory B cells in AAV patients has previously been reported[160-162, 168]. The discrepancy between the studies could possibly be related to differences in medication or disease activity between the cohorts.

The percentage of circulating plasmablasts and plasma cells ($CD27^{+}CD38^{++}$ B cells) has been shown to be increased in GPA patients with future relapse[161]. Increased frequency of circulating $CD27^{+}CD38^{++}$ B cells during remission could therefore be a potential marker to identify patients at risk of relapse. Also, in other autoimmune diseases such as SLE[169, 170] and IgG4-related disease[171], the plasmablast frequency has been reported to be related to disease activity. Here, we report that AAV patients displayed expansion of plasmablasts compared to healthy controls (0.9% vs. 0.3%) (paper II, figure 1, and Figure 8 above), but there were no differences between patients in active versus inactive disease, or between the relapse versus no relapse group.

Decreased frequencies of B cells and transitional B cells in patients with a tendency to relapse

Patients in remission with a tendency to relapse had, compared to nonrelapsing patients, decreased frequencies of B cells (3.5% vs. 6.5%) and transitional B cells (0.1% vs. 1.1%) (paper II, figure 3, and Figure 9 below). This may be a result of immunosuppressive treatment as a higher proportion of patients in the relapse group had medication. In line with this, we show that patients with immunosuppressants had lower percentage and lower B cell count compared to the nonmedication group, whereas there was no difference between patients without immunosuppressants and healthy controls. In agreement with our findings, Appelgren et al. found that the prednisolone dose correlated negatively with the absolute number of B cells and the

number of naive and memory B cells (but not exhausted memory B cells)[168]. Treatment with cyclophosphamide has been shown to reduce B cell counts, albeit the rate and magnitude of the decrease are less than with rituximab[172].

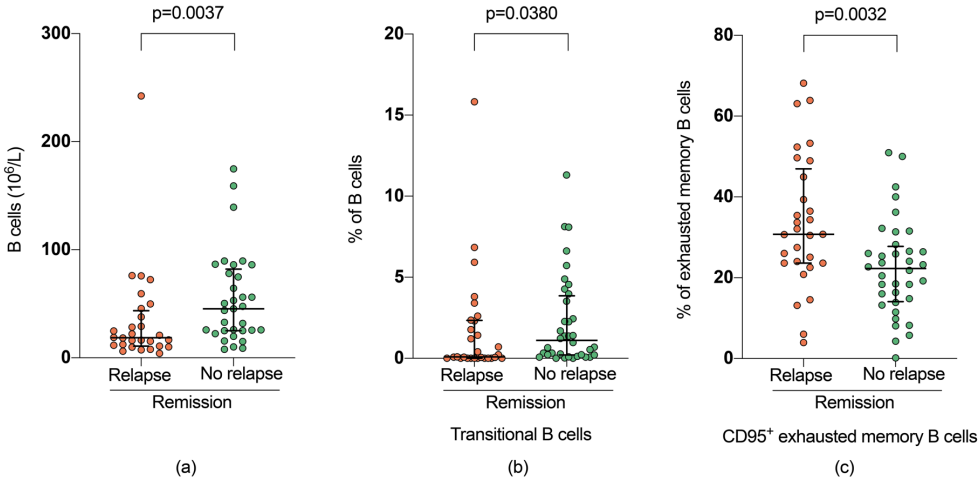


Figure 9. Comparisons of B cells and subsets between AAV patients in remission with and without a tendency to relapse. (a) Concentration of CD19⁺ B cells. Percentage of (b) transitional B cells (of CD19⁺ B cells), (c) CD95⁺ exhausted memory B cells (of exhausted memory B cells), in peripheral blood from patients with anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis (AAV) in remission, with and without tendency to relapse. Mann-Whitney *U* test was used to calculate the level of significance. Data are presented with medians and interquartile ranges. Reprinted from paper II, figure 3.

Patients in the relapse group also had increased frequency of activated exhausted memory B cells (30.8% vs. 22.3%). In SLE, a specific population of exhausted memory B cells has been demonstrated to be highly enriched, which implicates these autoreactive cells in autoimmune disease[173].

B cells can exert both regulatory and effector functions. Alterations in B cell subsets could translate to changes in the balance of these functions and may contribute to the autoantibody-driven inflammatory process, influence disease activity, and risk of relapse. However, the relative influence of disease activity and effect of medication on the B cell phenotype is difficult to separate in a complex autoimmune disease such as vasculitis.

Methotrexate suppresses monocytes in RA (paper III)

Patients with rheumatoid arthritis (RA) exhibit an increased risk of infections. The cause is likely a combination of the autoimmune nature of the disease and available pharmacological treatments[104, 105]. Therefore, immunization against vaccine-preventable diseases is important[105]. Methotrexate (MTX) impairs the antibody response to pneumococcal conjugate vaccine (PCV) in patients with arthritis, and the underlying mechanism is largely unknown. Here, we investigate the potential role of the innate immune system in the faltering antibody response following PCV immunization in RA patients treated with MTX. Phenotypes of circulating granulocytes and monocytes were analysed in 11 RA patients treated with MTX, 13 RA patients without disease modifying antirheumatic drug treatment (0DMARD), and 13 healthy controls. Peripheral blood samples were collected before and 7 days after vaccination. In addition, the MTX group was sampled before initiating treatment. Frequencies of granulocyte and monocyte subsets were determined using flow cytometry. Pneumococcal serotype-specific IgG concentrations of 11 serotypes included in 13-valent pneumococcal conjugate vaccine (PCV13) were analysed, right before and 4–6 weeks after vaccination.

MTX attenuates antibody response following pneumococcal vaccination

A positive antibody response (≥ twofold increase in ≥ 6 serotypes pre- to postvaccination) was seen in 90% of healthy controls, 87.5% of the 0DMARD group, and 56% of the MTX group. The composite antibody response, i.e. the sum of change in pneumococcal serotype-specific IgG concentrations (μg/mL), for the 11 capsular serotypes included in PCV13, pre- to postvaccination, is depicted in Figure 10 below (and paper III, figure 1). The composite antibody response was lower in the MTX group compared to healthy controls.

Two different but partially overlapping meta-analyses based on twelve and nine studies, respectively, concluded that MTX exposure diminishes the antibody response to pneumococcal vaccination[174, 175]. Park et al. demonstrated that holding MTX for four weeks (two weeks before and two weeks after vaccination or four weeks postvaccination) increased the response to quadrivalent seasonal influenza vaccination[176, 177]. This data further supports the direct role of MTX in decreased immune responsiveness in RA.

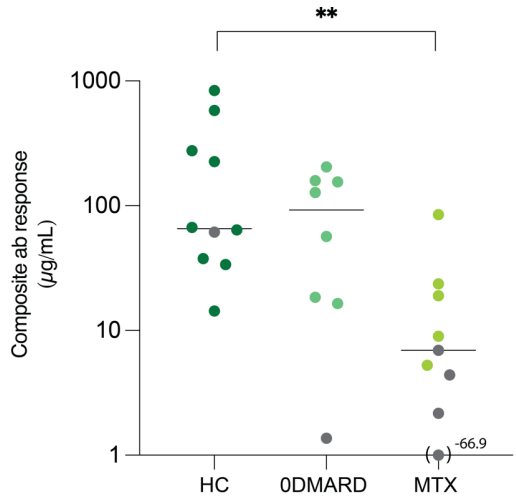


Figure 10. Composite antibody response in HC, RA 0DMARD, and RA MTX groups, after immunization with PCV13. Composite antibody response represents the sum of change in pneumococcal serotype-specific IgG concentrations (µg/mL), for 11 capsular serotypes included in PCV13, pre- to postvaccination. Nonresponders are depicted in grey and the remaining are responders (defined as ≥ twofold increase in antibody titers in ≥ 6 serotypes pre- to postvaccination). Kruskal-Wallis with Dunn's multiple comparisons test was used to calculate level of significance. Data are presented with medians. ** indicates p-value <0.01. ab: antibody; HC: healthy control; 0DMARD: without disease-modifying antirheumatic drug treatment; MTX: methotrexate; RA: rheumatoid arthritis; PCV13: 13-valent pneumococcal conjugate vaccine. Antibody titers were measured in 10 HC, 8 0DMARD and 9 MTX patients. Reprinted from paper III, figure 1.

Lower percentage of monocytes in nonresponders to pneumococcal conjugate vaccine in RA patients with MTX treatment

Antibody titers pre- and postvaccination were available for nine out of eleven patients with MTX treatment. Five patients displayed a positive antibody-response according to the definition (≥ twofold increase in antibody titers in ≥ 6 serotypes pre- to postvaccination) and four were nonresponders. There were no statistical differences between the groups regarding age, disease duration, disease activity, or MTX dose. After 6-12 weeks of MTX treatment (prevaccination), the percentage and concentration of monocytes were lower in nonresponders. The lower percentage of monocytes in nonresponders remained after vaccination (paper III, figure 3 and Figure 11 below).

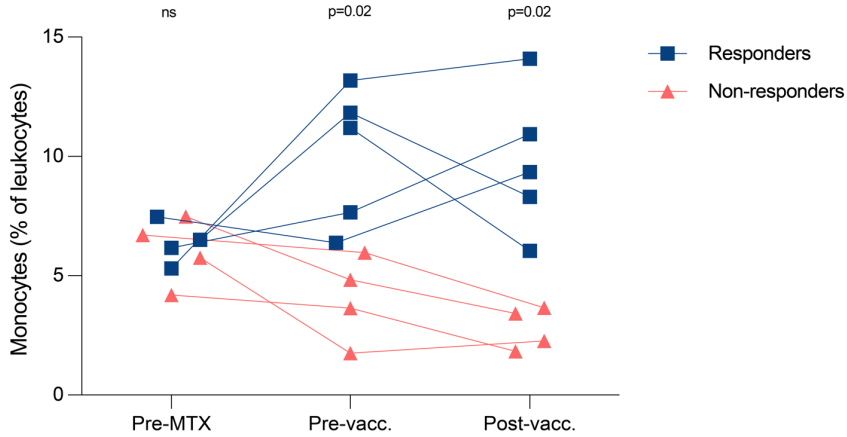


Figure 11. Comparison of monocytes (% of leukocytes), before initiation of methotrexate (MTX) treatment (Pre-MTX), with MTX treatment for 6-12 weeks and before vaccination (Pre-vacc.), and 6-7 days after administration of 13-valent pneumococcal conjugate vaccine (Post-vacc.), in peripheral blood from rheumatoid arthritis patients, sorted in responders and nonresponders to the vaccine. Positive antibody response was defined as an antibody response ratio (ARR, i.e., the ratio of post- to prevaccination antibody levels) ≥2, in >50% of serotypes. Flow cytometry data was not available for one patient (responder) Pre-MTX. Reprinted from paper III, figure 3.

Monocytes, macrophages, and granulocytes are important to the innate response to vaccine antigens and adjuvant, and are necessary to provide an effective adaptive immune response[110, 178, 179]. We noticed a lower frequency of classical monocytes and a higher frequency of inflammatory monocytes in nonresponders following MTX treatment; however, these changes were not significant. Several studies indicate an inverse correlation between frequency of inflammatory monocytes and antibody response, possibly via a defect of T cell help to B cells[180]. Further, Mitchell et al. have shown in murine models and in vitro that interrupting inflammatory monocyte recruitment to lymph nodes leads to enhanced cellular and humoral immune responses to vaccination[181].

Moreover, prior to start of MTX treatment there were tendencies of higher C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) in nonresponders compared to responders, and following MTX treatment (at the time of vaccination), ESR was higher in nonresponders (paper III, table S6). Possibly, the higher level of inflammation could contribute to the decreased antibody response in nonresponders. In support of this, Nakaya et al. have in an extensive study of gene signatures related to immunogenicity of influenza vaccination showed that baseline genetic signatures of monocyte inflammation were negatively correlated to antibody responses at one month. They reason that inflammation prevaccination might be unfavourable to the vaccine-induced antibody response[182].

Granulocytes and monocytes in ANCA-associated vasculitis (paper IV)

Here, we explored the relation between granulocyte/monocyte subsets and disease activity and tendency to relapse in AAV patients. A cohort of 105 patients with granulomatosis with polyangiitis (GPA) or microscopic polyangiitis (MPA) and 126 healthy controls were included. Clinical and laboratory data were collected, including disease activity, tendency to relapse and pharmacological treatment.

Using flow cytometry, circulating eosinophils, basophils, neutrophils, and monocytes were assessed. The monocytes were subdivided into classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{-}CD16^{+}$) monocytes. Mature ($CD16^{high}$) or newly released ($CD16^{dim}$) neutrophils were defined, as well as the frequency of $CD177^{+}$ neutrophils.

Increased frequency of neutrophils and intermediate monocytes in AAV

Neutrophils are generally the first recruited cells to an inflammatory site[183], followed by monocytes. In AAV patients, there were increased frequencies of early released $CD16^{+}$ neutrophils, mature $CD16^{high}$ neutrophils and as well $CD177^{+}$ neutrophils compared to healthy controls (paper IV, table 2). These results are in line with a previous study, indicating that AAV patients have skewed neutrophil and monocyte profiles[7].

Granulocytes and monocytes in active disease and remission

The association between monocytes and disease activity has been debated. To determine frequencies of granulocytes in relation to disease activity, 23 patients who had been sampled repeatedly, with a least one blood sample during the active disease period and one during remission, were selected. The analysis included the last collected sample, and the one prior to that from either remission or active disease depending on the disease activity of the last sample. No specific time interval between the two samples was considered. We found that the frequency of mature $CD16^{high}$ neutrophils increased, and the frequencies of total and intermediate monocytes decreased in active disease (paper IV, figure 3 and Figure 12 below). This could be related to recruitment of monocytes to the site of inflammation. A similar phenomenon was observed in MPA patients with a tendency to relapse, indicating that they have an ongoing low-grade inflammation.

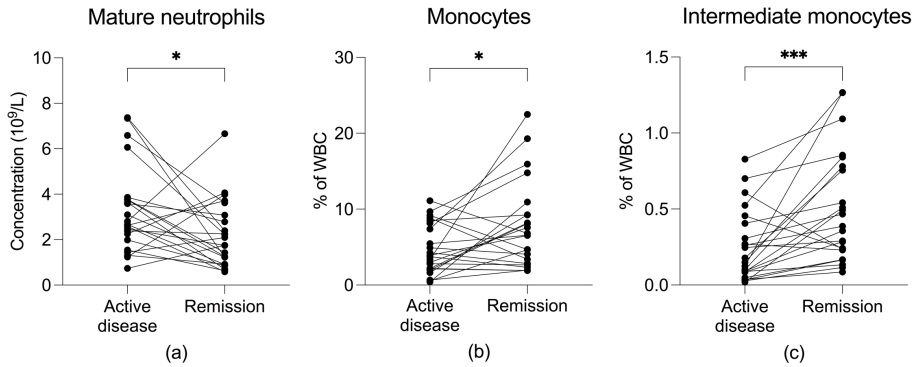


Figure 12. Concentration of (a) mature ($CD16^{high}$) neutrophils and frequencies of (b) total monocytes and (c) intermediate ($CD14^{++}CD16^{+}$) monocytes, in 23 patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) in active disease and remission. Wilcoxon matched-pairs signed rank test was used to calculate level of significance. * and *** indicate p-value <0.05 and <0.001, respectively. WBC: white blood cell. Adapted from paper IV, figure 3.

Neutrophils have been extensively studied in AAV and here we could confirm previous results showing that AAV patients have increased frequency of $CD177^{+}$ neutrophils[7] and that the neutrophil counts are increased during active disease. CD177 is co-expressed with PR3 on the surface of neutrophils[184] and have been associated with increased disease activity in AAV and poor clinical outcome[185-187].

Granulocytes and monocytes in relation to relapse tendency

Tendency to relapse (Ttr) was defined by the recurrence of disease after complete remission, when the patient had received standard of care and at least one year of follow-up was completed. Patients in need of increased dose of immunosuppressive treatment and Birmingham Vasculitis Activity Score version 3 (BVAS3) >1 were included in the Ttr group. AAV patients who fulfilled the criteria for evaluation of tendency to relapse (Ttr) were divided in two groups, the Ttr group (n=47) and the No Ttr group (n=34). Ttr patients had decreased percentage of eosinophils and increased frequencies of $CD16^{high}$ and $CD177^{+}$ neutrophils (paper IV, figure 4 a-c and Figure 13 below).

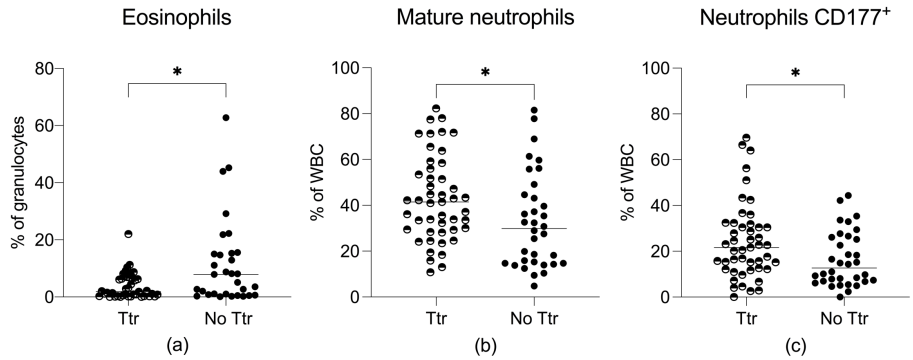


Figure 13. Frequencies of (a) eosinophils, (b) mature ($CD16^{high}$) neutrophils and (c) $CD177^{+}$ neutrophils, in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) patients with or without tendency to relapse (Ttr). Mann-Whitney *U* test was used to calculate level of significance. Data are presented with medians. * indicates p-value <0.05. Ttr: tendency to relapse; No Ttr: no tendency to relapse; WBC: white blood cell. Adapted from paper IV, figure 4.

When dividing the patients based on disease phenotype, GPA patients with Ttr displayed higher frequencies of mature and $CD177^{+}$ neutrophils, whilst MPA patients with Ttr had decreased frequency of intermediate monocytes, as compared to the GPA and MPA No Ttr group, respectively (paper IV, figure 4 d-h).

Conclusions

The main conclusions of this thesis are:

Paper I — Thirty-eight new and five known sequence variants associated with IgA, IgG or IgM levels or with composite immunoglobulin traits. These associations were related to 32 genomic loci and included rare variants with strong effects. The results present new insights into sequence variants that influence immunoglobulin levels in humans and may provide deeper knowledge of the mechanisms involved in the regulation of the humoral immune system.

Paper II — Patients with ANCA-associated vasculitis (AAV) exhibited decreased frequencies of B cells and transitional B cells, and increased frequencies of switched memory B cells, plasmablasts and activated B cells in peripheral blood. Further, patients with a tendency to relapse had decreased frequencies of B cells and transitional B cells, as well as a higher proportion of activated exhausted memory B cells, compared to patients without tendency to relapse. These alterations could contribute to the autoantibody-driven inflammatory process in AAV.

Paper III — Methotrexate-treated nonresponders to pneumococcal conjugate vaccine displayed lower frequency and concentration of monocytes compared to responders. Monocyte frequency in peripheral blood could have the potential to act as a biomarker to identify future nonresponders to pneumococcal vaccination in methotrexate-treated RA patients.

Paper IV — AAV patients displayed a skewing of different neutrophil and monocyte subpopulations that associated with disease activity and tendency to relapse. These changes may contribute to the inflammatory process and might be used as biomarkers for relapse prediction.

Future perspectives

Identification of sequence variants influencing immunoglobulin levels (paper I)

The discovered GWAS associations explained 4.3-8.7% of the variance in the nine Ig traits, which means that a large majority of the variance is dependent on other genetic associations or factors. In support of that, many genes with clear roles in class switching (such as *CD40*, *IRF4*, *AICDA*, *XBP1*, *BATF3* and *NFIL3*) were absent from associated loci, even though several proxy traits were designed to capture these aspects. This suggests that these composite immunoglobulin proxy traits do not capture all the genetic variability that can affect class switching of immunoglobulins. In the study, candidate genes and mechanisms underlying the identified associations were identified using bioinformatic and functional studies. However, as pointed out in paper I, further experimental validation work and analyses are needed to identify the disease-causal variants, the genes they regulate and the affected cell types driving the phenotype for most of the loci.

Increased frequencies of switched memory B cells and plasmablasts in peripheral blood from patients with ANCA-associated vasculitis (paper II)

An important limitation for this study was the possible influence of medication on the immune cells, and we did demonstrate that patients with pharmacological treatment had a lower percentage of B cells compared to patients without medications. In a future study, age- and gender-matched therapy controls would be required to elucidate whether the alterations in B cell subsets are specifically related to the immunosuppressive treatment or the autoimmune disease (or both). In addition to the decrease in B cell concentration and frequency, the most interesting and clear finding was the lower frequency of transitional B cells. Relapsing AAV patients displayed a ten-fold lower frequency of this subset as compared to nonrelapsing patients. This could potentially affect the balance of anti- and proinflammatory cytokines as transitional B cells can produce the anti-inflammatory cytokine IL-10. Measurement of circulating cytokine levels could be included in future studies.

Methotrexate treatment suppresses monocytes in nonresponders to pneumococcal conjugate vaccine in rheumatoid arthritis patients (paper III)

The timing of sampling following vaccination is very important for analyses of the rapidly reacting innate immune system. In the study, the sampling time following

vaccination was not optimal. Preferably, several sampling times should be included during the first days following vaccination to follow the response of the different immune cells involved. This could be combined with a broad analysis of circulating cytokines at these early timepoints. In addition, we used serotype-specific pneumococcal antibody response ratio (post- to prevaccination) to determine vaccine responsiveness. Analysis of the T cell response to the vaccine, as well as opsonophagocytosis, would have added information complementing the serotype-specific antibody response. The most interesting finding of the study was the suppressive effect of MTX on monocytes in future nonresponders to PCV immunization. This should clearly be reproduced in a larger cohort. In such study, the next step would be to explore the underlying mechanisms. Such study could include earlier sampling timepoints, extensive coverage of circulating innate immune cells, as well as relevant signalling molecules. In addition, knowledge about MTX-induced tissue alterations of cell populations of the innate and adaptive immune system could contribute to the understanding of changes found in circulating immune cells. This could potentially be explored in animal experiments.

Disease activity and tendency to relapse in ANCA-associated vasculitis are reflected in neutrophil and intermediate monocyte frequencies (paper IV)

Similar to the study of AAV patients in paper II, the possible influence of medication on the immune cell distribution was a possible confounder, especially in comparisons between patients and healthy controls. In a future study, sampling at diagnosis, prior to start of medication could help validate the noted alterations in intermediate monocytes and neutrophils, also in the paired within-patient comparison of active disease vs. remission. In addition, determination of key circulating signalling molecules, biomarkers, and potentially functional assays could help unravel the underlying mechanisms for the alterations in the specific cell populations. We demonstrated that rituximab treatment was associated with changes in the monocyte population and further investigations will be needed to evaluate the correlation to treatment response. Moreover, the increased frequencies of mature and $CD177^{+}$ neutrophils in GPA patients with a tendency to relapse can potentially be used as biomarkers for relapse prediction in this patient group.

Summary in Swedish

Immunsystemet har till uppgift att skydda oss mot patogener som till exempel bakterier, virus, giftiga ämnen och annat kroppsfrämmande. Det kan grovt delas in i det medfödda och det förvärvade immunförsvaret. Det medfödda kallas även det ospecifika immunförsvaret och inbegriper förutom celler och signalämnen i blod och vävnader, andra skyddsmekanismer såsom hud, slemhinnor och magsyra. Det medfödda immunförsvaret är den första linjens försvar och agerar snabbt men inte lika kraftfullt och specifikt som det förvärvade immunförsvaret. Det förvärvade immunförsvaret kallas även det adaptiva (föränderliga) immunförsvaret och fortsätter att utvecklas hela livet. Första gången det adaptiva immunförsvaret möter en ny patogen behöver det tid för att nå full potential. Däremot utvecklar det ett immunologiskt minne som gör att om vi åter utsätts för samma patogen slår det till mycket snabbare och med full kraft direkt. Celler inom det adaptiva immunförsvaret utgörs av vita blodkroppar som kallas T- och B-celler. B-cellerna är viktiga för produktionen av så kallade antikroppar. De är proteiner som hjälper till att bekämpa kroppsfrämmande ämnen. Antikroppar kallas även immunglobuliner (Ig) och finns i fem olika klasser. I blod och slemhinnor finns framför allt klasserna IgG, IgM och IgA.

I den här avhandlingen ingår fyra projekt. I det första projektet undersökte vi sambandet mellan olika genvarianter i arvsmassan och nivån av immunglobuliner i blodet. Över 19 000 individer från den allmänna befolkningen ingick i studien. Vi fann 38 nya och fem kända varianter med association till IgG, IgM eller IgA nivåerna eller kombinationer av dessa. Dessa genvarianter är kopplade till platser i arvsmassan där det också finns varianter som har koppling till autoimmuna sjukdomar, blodcancer och blodcellers utveckling. Resultaten ger nya insikter om gener som påverkar antikroppsnivåerna i blodet. Det kan också leda till mer kunskap om de mekanismer i det adaptiva immunsystemet som alstrar antikroppar.

De båda delarna av immunsystemet är nära sammanflätade och samarbetar kontinuerligt. Det finns inbyggda mekanismer som gör att kroppen inte angriper egen vävnad, men trots det kan felaktigheter uppstå som gör att vårt immunförsvar angriper kroppens egna celler, vilket kan leda till så kallad autoimmun sjukdom.

I det andra projektet undersökte vi andelen B-celler i blod från patienter med den ovanliga men allvarliga sjukdomen ANCA-associerad vaskulit (blodkärls-inflammation). ANCA-associerad vaskulit (AAV) drabbar framför allt små blodkärl

och många patienter får uttalade njurskador, men även andra organ kan drabbas. Förr var det en dödlig sjukdom, men med dagens behandlingar har det blivit en kronisk sjukdom som går i skov med begränsad sjuklighet. Att vissa drabbas av AAV beror sannolikt på flera faktorer, t.ex. arv, miljöfaktorer och immunförsvaret. Vid AAV bildas autoantikroppar som angriper kroppsegna ämnen. De kallas ANCA (Anti-Neutrophil Cytoplasmic Antibody) och binder till två typer av vita blodkroppar (neutrofiler och monocyter). Vi fann att patienter med AAV har lägre andel B-celler jämfört med friska individer (blodgivare), men att de har en högre andel aktiva B-celler än friska. Patienter med skovbenägenhet hade lägre andel B-celler och skillnader i undergrupper av B-celler jämfört med de som inte hade skovbenägenhet. Möjligen kan förändringarna i B-cellerna vara en bidragande orsak till den autoantikropps-drivna inflammationen i AAV.

I det fjärde projektet studerade vi om det finns en koppling mellan skovbenägenhet i AAV och de vita blodkropparna granulocyter och monocyter, som är celler inom det medfödda immunförsvaret. Granulocyter utgörs av tre celltyper; neutrofiler, eosinofiler och basofiler. Neutrofilerna utgör mer än hälften av alla vita blodkroppar i blodet. Deras främsta uppgift är att fagocytera (äta upp) och eliminera patogener såsom bakterier och svampar. De tros också ha en viktig roll i AAV. Neutrofilerna finns i och runt de inflammerade kärlväggarna. Autoantikropparna vid AAV aktiverar neutrofilerna vilket i sin tur leder till ökad inflammation. Monocyternas roll i AAV är inte lika känd, men de har påvisats i kärl i påverkade organ såsom njurar och lungor. När skador uppstår vid AAV kommer monocyter att rekryteras och mogna till så kallade makrofager i vävnaden. Vi fann att patienter i aktiv sjukdom har högre koncentration av mogna neutrofiler och minskad andel monocyter i blodet jämfört med de som är i remission (tillstånd utan sjukdomssymtom). Patienter med skovbenägenhet hade ökad andel mogna neutrofiler. Dessa förändringar skulle kunna bidra till inflammationen och möjligen kunna användas för att förutspå ett skov.

I det tredje projektet studerade vi andelen granulocyter och monocyter i blod från patienter med en annan autoimmun sjukdom, ledgångsreumatism. Tidigare studier har visat att patienter med ledgångsreumatism (reumatisk artrit, RA) som behandlas med läkemedlet metotrexat får ett dåligt antikroppssvar efter vaccination mot bland annat pneumokocker. Orsaken till detta är inte känd. RA är en kronisk autoimmun sjukdom med ledinflammation som ofta börjar i små leder i händer och fötter. Obehandlad RA leder i de allra flesta fall till destruktion av ben och brosk, som i sin tur ger uttalade funktionsnedsättningar, smärta och stelhet. Orsaken till RA är inte känd. Arv och miljöfaktorer som t.ex. rökning har betydelse för sjukdomsutvecklingen. Tidig diagnos och tidigt påbörjad antireumatisk behandling med långverkande sjukdomsmodifierande anti-reumatiska läkemedel (Disease-Modifying AntiRheumatic Drugs, DMARDs) är avgörande för prognosen. Metotrexat är ett sådant läkemedel. Metotrexat i hög dos ges vid cancer för att bromsa cancercellernas tillväxt medan låg dos metotrexat dämpar ledinflammation

och är förstahandsbehandling vid RA. Det är viktigt att RA patienter vaccineras eftersom de har en ökad risk för infektioner, delvis beroende på den autoimmuna sjukdomen men även som en konsekvens av läkemedelsbehandlingen. Pneumokocker är den vanligaste orsaken till bakteriella luftvägsinfektioner såsom lunginflammation, öroninflammation och bihåleinflammation, men kan även orsaka hjärnhinneinflammation och blodförgiftning.

I vaccin är patogenen som orsakar sjukdom försvagad för att inte orsaka sjukdom, men tillräcklig reaktion för att stimulera immunförsvaret att bl.a. bilda ett så kallat immunologiskt minne. Vaccinet får immunförsvaret att tillverka de celler och antikroppar som behövs för att motverka just den sjukdomen. Både det medfödda och adaptiva immunförsvaret är involverat i vaccinationssvaret. Monocyter och granulocyter är viktiga för det medfödda immunförsvarets svar på vaccin och nödvändiga för ett effektivt adaptivt immunförsvar. Det adaptiva immunförsvaret i sin tur bildar antikroppar och vita blodkroppar som har ett minne. Det är minnescellerna och kvarvarande antikroppar som gör att vi inte blir sjuka eller enbart får milda symtom vid nästa exponering för samma patogen. I vår studie med nio patienter som behandlades med metotrexat, fick fem ett bra antikroppssvar medan fyra patienter fick ett dåligt antikroppsvar mot pneumokocker. Före behandling med metotrexat hade alla nio patienter lika stor andel monocyter i blodet. Under behandlingen med metotrexat fick de som senare inte svarade på vaccinet lägre koncentration och andel monocyter jämfört med de som svarade på vaccinet. Möjligen skulle andelen och/eller koncentrationen monocyter kunna vara vägledande för att identifiera vilka som kommer att få ett skyddande antikroppsvar efter vaccination i denna patientgrupp. Fyndet behöver bekräftas i studier med fler patienter, och mekanismen bakom undersökas.

Acknowledgements

I would like to express my warmest gratitude to all of those who have contributed and supported me in the work on this thesis. Especially I would like to thank:

All the participating patients and healthy donors who contributed to these studies.

Åsa Johansson, my supervisor, for sharing your knowledge and extensive experience in immunology and flow cytometry, and for being an inspiring researcher.

Thomas Hellmark, my co-supervisor, for giving me the opportunity to increase my understanding of ANCA-associated vasculitis, and for valuable guidance.

Sofia Smargianaki, my dear colleague at the Department of Clinical Immunology and Transfusion Medicine and co-author, for great collaboration.

Annica Andreasson, for sharing long-term hands-on experience in flow cytometry and fruitful discussions. Thank you for always being positive and helpful.

Åsa Pettersson, for performing laboratory work with diligence and carefulness.

Meliha Kapetanovic, for giving me the opportunity to work with the exciting rheumatic arthritis material and always responding swiftly and with acuity.

All additional co-authors for your important contributions to our work.

All members of the research group, past and present, for a friendly and inviting research environment.

Ann Jönsson, section head at the Department of Clinical Immunology and Transfusion Medicine, for being supportive of research and allocating time for my PhD studies.

All colleagues and co-workers at the Department of Clinical Immunology and Transfusion Medicine, for making it possible for me to combine clinical and scientific work.

Lilly and Lennart, my parents, for always being there, ready to help and support, and for your love. Wiveka and Mats, my parents-in-law, for supporting and being proud of my academic aspirations.

Eskil, my husband, for your endless encouragement, indefatigable enthusiasm and unwavering love. Saga, Vidar and Alva, our beloved children.

References

1. Cooper MD, Peterson RD, Good RA. Delineation of the thymic and bursal lymphoid systems in the chicken. Nature 1965;205:143-146.
2. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. Allergy Asthma Clin Immunol 2018;14:49.
3. Spiering MJ. Primer on the Immune System. Alcohol Res 2015;37:171-175.
4. Herrero-Cervera A, Soehnlein O, Kenne E. Neutrophils in chronic inflammatory diseases. Cell Mol Immunol 2022;19:177-191.
5. Kessenbrock K, Krumbholz M, Schönermarck U, et al. Netting neutrophils in autoimmune small-vessel vasculitis. Nat Med 2009;15:623-625.
6. Xiao H, Schreiber A, Heeringa P, Falk RJ, Jennette JC. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. Am J Pathol 2007;170:52-64.
7. Johansson ÅCM, Ohlsson S, Pettersson Å, et al. Impaired phagocytosis and reactive oxygen species production in phagocytes is associated with systemic vasculitis. Arthritis Research & Therapy 2016;18:92.
8. Ravin KA, Loy M. The Eosinophil in Infection. Clin Rev Allergy Immunol 2016;50:214-227.
9. Affandi AJ, Olesek K, Grabowska J, et al. CD169 Defines Activated CD14(+) Monocytes With Enhanced CD8(+) T Cell Activation Capacity. Front Immunol 2021;12:697840.
10. Kratofil RM, Kubes P, Deniset JF. Monocyte Conversion During Inflammation and Injury. Arterioscler Thromb Vasc Biol 2017;37:35-42.
11. Ziegler-Heitbrock L, Ancuta P, Crowe S, et al. Nomenclature of monocytes and dendritic cells in blood. Blood 2010;116:e74-80.
12. Cormican S, Griffin MD. Human Monocyte Subset Distinctions and Function: Insights From Gene Expression Analysis. Front Immunol 2020;11:1070.
13. Sampath P, Moideen K, Ranganathan UD, Bethunaickan R. Monocyte Subsets: Phenotypes and Function in Tuberculosis Infection. Front Immunol 2018;9:1726.
14. Kapellos TS, Bonaguro L, Gemünd I, et al. Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases. Front Immunol 2019;10:2035.
15. Vegting Y, Vogt L, Anders HJ, de Winther MPJ, Bemelman FJ, Hilhorst ML. Monocytes and macrophages in ANCA-associated vasculitis. Autoimmun Rev 2021;20:102911.
16. Cabeza-Cabrerizo M, Cardoso A, Minutti CM, Pereira da Costa M, Reis e Sousa C. Dendritic Cells Revisited. Annu Rev Immunol 2021;39:131-166.
17. Heath WR, Kato Y, Steiner TM, Caminschi I. Antigen presentation by dendritic cells for B cell activation. Curr Opin Immunol 2019;58:44-52.
18. Zanna MY, Yasmin AR, Omar AR, et al. Review of Dendritic Cells, Their Role in Clinical Immunology, and Distribution in Various Animal Species. Int J Mol Sci 2021;22.

19. Hirayama D, Iida T, Nakase H. The Phagocytic Function of Macrophage-Enforcing Innate Immunity and Tissue Homeostasis. Int J Mol Sci 2017;19.
20. Zhang C, Yang M, Ericsson AC. Function of Macrophages in Disease: Current Understanding on Molecular Mechanisms. Front Immunol 2021;12:620510.
21. Vivier E, Artis D, Colonna M, et al. Innate Lymphoid Cells: 10 Years On. Cell 2018;174:1054-1066.
22. Wu L, Van Kaer L. Natural killer T cells in health and disease. Front Biosci (Schol Ed) 2011;3:236-251.
23. Rampoldi F, Ullrich L, Prinz I. Revisiting the Interaction of γδ T-Cells and B-Cells. Cells 2020;9.
24. Deseke M, Prinz I. Ligand recognition by the γδ TCR and discrimination between homeostasis and stress conditions. Cell Mol Immunol 2020;17:914-924.
25. Christie SM, Fijen C, Rothenberg E. V(D)J Recombination: Recent Insights in Formation of the Recombinase Complex and Recruitment of DNA Repair Machinery. Front Cell Dev Biol 2022;10:886718.
26. Krangel MS. Mechanics of T cell receptor gene rearrangement. Curr Opin Immunol 2009;21:133-139.
27. Bassing CH, Swat W, Alt FW. The mechanism and regulation of chromosomal V(D)J recombination. Cell 2002;109 Suppl:S45-55.
28. Ruterbusch M, Pruner KB, Shehata L, Pepper M. In Vivo CD4(+) T Cell Differentiation and Function: Revisiting the Th1/Th2 Paradigm. Annu Rev Immunol 2020;38:705-725.
29. Butcher MJ, Zhu J. Recent advances in understanding the Th1/Th2 effector choice. Fac Rev 2021;10:30.
30. Jankovic D, Feng CG. CD4(+) T Cell Differentiation in Infection: Amendments to the Th1/Th2 Axiom. Front Immunol 2015;6:198.
31. Zhu X, Zhu J. CD4 T Helper Cell Subsets and Related Human Immunological Disorders. Int J Mol Sci 2020;21.
32. Takeuchi A, Saito T. CD4 CTL, a Cytotoxic Subset of CD4(+) T Cells, Their Differentiation and Function. Front Immunol 2017;8:194.
33. Cooper MD, Alder MN. The evolution of adaptive immune systems. Cell 2006;124:815-822.
34. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. Blood 2008;112:1570-1580.
35. Sanz I, Wei C, Jenks SA, et al. Challenges and Opportunities for Consistent Classification of Human B Cell and Plasma Cell Populations. Front Immunol 2019;10:2458.
36. Glass DR, Tsai AG, Oliveria JP, et al. An Integrated Multi-omic Single-Cell Atlas of Human B Cell Identity. Immunity 2020;53:217-232.e215.
37. Kwak K, Akkaya M, Pierce SK. B cell signaling in context. Nat Immunol 2019;20:963-969.
38. Treanor B. B-cell receptor: from resting state to activate. Immunology 2012;136:21-27.
39. Roth DB. V(D)J Recombination: Mechanism, Errors, and Fidelity. Microbiol Spectr 2014;2.
40. Schatz DG, Swanson PC. V(D)J recombination: mechanisms of initiation. Annu Rev Genet 2011;45:167-202.
41. Cyster JG, Allen CDC. B Cell Responses: Cell Interaction Dynamics and Decisions. Cell 2019;177:524-540.

42. Eisen HN. Affinity enhancement of antibodies: how low-affinity antibodies produced early in immune responses are followed by high-affinity antibodies later and in memory B-cell responses. Cancer Immunol Res 2014;2:381-392.
43. Lightman SM, Utley A, Lee KP. Survival of Long-Lived Plasma Cells (LLPC): Piecing Together the Puzzle. Front Immunol 2019;10:965.
44. Sebina I, Pepper M. Humoral immune responses to infection: common mechanisms and unique strategies to combat pathogen immune evasion tactics. Curr Opin Immunol 2018;51:46-54.
45. Kurosaki T, Kometani K, Ise W. Memory B cells. Nat Rev Immunol 2015;15:149-159.
46. Schroeder HW, Jr., Cavacini L. Structure and function of immunoglobulins. J Allergy Clin Immunol 2010;125:S41-52.
47. Murin CD. Considerations of Antibody Geometric Constraints on NK Cell Antibody Dependent Cellular Cytotoxicity. Front Immunol 2020;11:1635.
48. Nimmerjahn F, Ravetch JV. Fcgamma receptors as regulators of immune responses. Nat Rev Immunol 2008;8:34-47.
49. Schwartz RH. Historical overview of immunological tolerance. Cold Spring Harb Perspect Biol 2012;4:a006908.
50. Kamradt T, Mitchison NA. Tolerance and autoimmunity. N Engl J Med 2001;344:655-664.
51. Klein L, Kyewski B, Allen PM, Hogquist KA. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). Nat Rev Immunol 2014;14:377-391.
52. van Meerwijk JP, Marguerat S, Lees RK, Germain RN, Fowlkes BJ, MacDonald HR. Quantitative impact of thymic clonal deletion on the T cell repertoire. J Exp Med 1997;185:377-383.
53. Nagamine K, Peterson P, Scott HS, et al. Positional cloning of the APECED gene. Nat Genet 1997;17:393-398.
54. Esensten JH, Helou YA, Chopra G, Weiss A, Bluestone JA. CD28 Costimulation: From Mechanism to Therapy. Immunity 2016;44:973-988.
55. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature 2017;541:321-330.
56. Ferreira LMR, Muller YD, Bluestone JA, Tang Q. Next-generation regulatory T cell therapy. Nat Rev Drug Discov 2019;18:749-769.
57. Park JH, Lee KH, Jeon B, et al. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome: A systematic review. Autoimmun Rev 2020;19:102526.
58. Arpaia N, Green JA, Moltedo B, et al. A Distinct Function of Regulatory T Cells in Tissue Protection. Cell 2015;162:1078-1089.
59. Bluestone JA, Anderson M. Tolerance in the Age of Immunotherapy. N Engl J Med 2020;383:1156-1166.
60. Nemazee D. Mechanisms of central tolerance for B cells. Nat Rev Immunol 2017;17:281-294.
61. Winkler TH, Mårtensson IL. The Role of the Pre-B Cell Receptor in B Cell Development, Repertoire Selection, and Tolerance. Front Immunol 2018;9:2423.
62. Cashman KS, Jenks SA, Woodruff MC, et al. Understanding and measuring human B-cell tolerance and its breakdown in autoimmune disease. Immunol Rev 2019;292:76-89.
63. Müller J, Nitschke L. The role of CD22 and Siglec-G in B-cell tolerance and autoimmune disease. Nat Rev Rheumatol 2014;10:422-428.
64. Platt JL, Garcia de Mattos Barbosa M, Cascalho M. The five dimensions of B cell tolerance. Immunol Rev 2019;292:180-193.

65. Catalán D, Mansilla MA, Ferrier A, et al. Immunosuppressive Mechanisms of Regulatory B Cells. Front Immunol 2021;12:611795.
66. Chekol Abebe E, Asmamaw Dejenie T, Mengie Ayele T, Dagnew Baye N, Agegnehu Teshome A, Tilahun Muche Z. The Role of Regulatory B Cells in Health and Diseases: A Systemic Review. J Inflamm Res 2021;14:75-84.
67. Khan AR, Hams E, Floudas A, Sparwasser T, Weaver CT, Fallon PG. PD-L1hi B cells are critical regulators of humoral immunity. Nat Commun 2015;6:5997.
68. Krainer J, Siebenhandl S, Weinhäusel A. Systemic autoinflammatory diseases. J Autoimmun 2020;109:102421.
69. Geetha D, Jefferson JA. ANCA-Associated Vasculitis: Core Curriculum 2020. Am J Kidney Dis 2020;75:124-137.
70. Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum 2013;65:1-11.
71. Jennette JC, Nachman PH. ANCA Glomerulonephritis and Vasculitis. Clin J Am Soc Nephrol 2017;12:1680-1691.
72. Austin K, Janagan S, Wells M, Crawshaw H, McAdoo S, Robson JC. ANCA Associated Vasculitis Subtypes: Recent Insights and Future Perspectives. J Inflamm Res 2022;15:2567-2582.
73. Cornec D, Cornec-Le Gall E, Fervenza FC, Specks U. ANCA-associated vasculitis - clinical utility of using ANCA specificity to classify patients. Nat Rev Rheumatol 2016;12:570-579.
74. Mohammad AJ. An update on the epidemiology of ANCA-associated vasculitis. Rheumatology (Oxford) 2020;59:iii42-iii50.
75. Mukhtyar C, Lee R, Brown D, et al. Modification and validation of the Birmingham Vasculitis Activity Score (version 3). Ann Rheum Dis 2009;68:1827-1832.
76. Xiao H, Hu P, Falk RJ, Jennette JC. Overview of the Pathogenesis of ANCA-Associated Vasculitis. Kidney Dis (Basel) 2016;1:205-215.
77. Kitching AR, Anders HJ, Basu N, et al. ANCA-associated vasculitis. Nat Rev Dis Primers 2020;6:71.
78. Oleinika K, Mauri C, Salama AD. Effector and regulatory B cells in immune-mediated kidney disease. Nat Rev Nephrol 2019;15:11-26.
79. Lepse N, Abdulahad WH, Kallenberg CG, Heeringa P. Immune regulatory mechanisms in ANCA-associated vasculitides. Autoimmun Rev 2011;11:77-83.
80. Rincón-Arévalo H, Sanchez-Parra CC, Castaño D, Yassin L, Vásquez G. Regulatory B Cells and Mechanisms. Int Rev Immunol 2016;35:156-176.
81. Jennette JC, Falk RJ. Pathogenesis of antineutrophil cytoplasmic autoantibody-mediated disease. Nat Rev Rheumatol 2014;10:463-473.
82. Jennette JC. Implications for pathogenesis of patterns of injury in small- and medium-sized-vessel vasculitis. Cleve Clin J Med 2002;69 Suppl 2:Sii33-38.
83. Jennette JC, Wilkman AS, Falk RJ. Anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis and vasculitis. Am J Pathol 1989;135:921-930.
84. Xiao H, Dairaghi DJ, Powers JP, et al. C5a receptor (CD88) blockade protects against MPO-ANCA GN. J Am Soc Nephrol 2014;25:225-231.
85. Samman KN, Ross C, Pagnoux C, Makhzoum JP. Update in the Management of ANCA-Associated Vasculitis: Recent Developments and Future Perspectives. Int J Rheumatol 2021;2021:5534851.

86. Wallace ZS, Miloslavsky EM. Management of ANCA associated vasculitis. Bmj 2020;368:m421.
87. Antonelou M, Michaëlsson E, Evans RDR, et al. Therapeutic Myeloperoxidase Inhibition Attenuates Neutrophil Activation, ANCA-Mediated Endothelial Damage, and Crescentic GN. J Am Soc Nephrol 2020;31:350-364.
88. McAdoo SP, Prendecki M, Tanna A, et al. Spleen tyrosine kinase inhibition is an effective treatment for established vasculitis in a pre-clinical model. Kidney Int 2020;97:1196-1207.
89. Springer JM, Kalot MA, Husainat NM, et al. Granulomatosis With Polyangiitis and Microscopic Polyangiitis: A Systematic Review and Meta-Analysis of Benefits and Harms of Common Treatments. ACR Open Rheumatol 2021;3:196-205.
90. Yates M, Watts R. ANCA-associated vasculitis. Clin Med (Lond) 2017;17:60-64.
91. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med 2011;365:2205-2219.
92. Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD, Tanasescu R. Extra-articular Manifestations in Rheumatoid Arthritis. Maedica (Bucur) 2010;5:286-291.
93. Widdifield J, Paterson JM, Huang A, Bernatsky S. Causes of Death in Rheumatoid Arthritis: How Do They Compare to the General Population? Arthritis Care Res (Hoboken) 2018;70:1748-1755.
94. Yap HY, Tee SZ, Wong MM, Chow SK, Peh SC, Teow SY. Pathogenic Role of Immune Cells in Rheumatoid Arthritis: Implications in Clinical Treatment and Biomarker Development. Cells 2018;7.
95. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010;62:2569-2581.
96. van Riel PL, Renskers L. The Disease Activity Score (DAS) and the Disease Activity Score using 28 joint counts (DAS28) in the management of rheumatoid arthritis. Clin Exp Rheumatol 2016;34:S40-s44.
97. Malmström V, Catrina AI, Klareskog L. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. Nat Rev Immunol 2017;17:60-75.
98. Holoshitz J. The rheumatoid arthritis HLA-DRB1 shared epitope. Curr Opin Rheumatol 2010;22:293-298.
99. Padyukov L. Genetics of rheumatoid arthritis. Semin Immunopathol 2022;44:47-62.
100. Hedström AK, Stawiarz L, Klareskog L, Alfredsson L. Smoking and susceptibility to rheumatoid arthritis in a Swedish population-based case-control study. Eur J Epidemiol 2018;33:415-423.
101. Almutairi KB, Nossent JC, Preen DB, Keen HI, Inderjeeth CA. The Prevalence of Rheumatoid Arthritis: A Systematic Review of Population-based Studies. J Rheumatol 2021;48:669-676.
102. Cross M, Smith E, Hoy D, et al. The global burden of rheumatoid arthritis: estimates from the global burden of disease 2010 study. Ann Rheum Dis 2014;73:1316-1322.
103. Myasoedova E, Davis J, Matteson EL, Crowson CS. Is the epidemiology of rheumatoid arthritis changing? Results from a population-based incidence study, 1985-2014. Ann Rheum Dis 2020;79:440-444.

104. Doran MF, Crowson CS, Pond GR, O'Fallon WM, Gabriel SE. Frequency of infection in patients with rheumatoid arthritis compared with controls: a population-based study. Arthritis Rheum 2002;46:2287-2293.
105. Furer V, Rondaan C, Heijstek MW, et al. 2019 update of EULAR recommendations for vaccination in adult patients with autoimmune inflammatory rheumatic diseases. Ann Rheum Dis 2020;79:39-52.
106. Shams S, Martinez JM, Dawson JRD, et al. The Therapeutic Landscape of Rheumatoid Arthritis: Current State and Future Directions. Front Pharmacol 2021;12:680043.
107. Cronstein BN, Aune TM. Methotrexate and its mechanisms of action in inflammatory arthritis. Nat Rev Rheumatol 2020;16:145-154.
108. Bullock J, Rizvi SAA, Saleh AM, et al. Rheumatoid Arthritis: A Brief Overview of the Treatment. Med Princ Pract 2018;27:501-507.
109. Riedel S. Edward Jenner and the history of smallpox and vaccination. Proc (Bayl Univ Med Cent) 2005;18:21-25.
110. Clem AS. Fundamentals of vaccine immunology. J Glob Infect Dis 2011;3:73-78.
111. El-Beyrouty C, Buckler R, Mitchell M, Phillips S, Groome S. Pneumococcal vaccination-A literature review and practice guideline update. Pharmacotherapy 2022.
112. Jackson LA, Gurtman A, van Cleeff M, et al. Influence of initial vaccination with 13-valent pneumococcal conjugate vaccine or 23-valent pneumococcal polysaccharide vaccine on anti-pneumococcal responses following subsequent pneumococcal vaccination in adults 50 years and older. Vaccine 2013;31:3594-3602.
113. Rappuoli R, De Gregorio E, Costantino P. On the mechanisms of conjugate vaccines. Proc Natl Acad Sci U S A 2019;116:14-16.
114. Lal G, Balmer P, Stanford E, Martin S, Warrington R, Borrow R. Development and validation of a nonaplex assay for the simultaneous quantitation of antibodies to nine Streptococcus pneumoniae serotypes. J Immunol Methods 2005;296:135-147.
115. Balmer P, Cant AJ, Borrow R. Anti-pneumococcal antibody titre measurement: what useful information does it yield? J Clin Pathol 2007;60:345-350.
116. Daly TM, Hill HR. Use and clinical interpretation of pneumococcal antibody measurements in the evaluation of humoral immune function. Clin Vaccine Immunol 2015;22:148-152.
117. Kapetanovic MC, Saxne T, Sjöholm A, Truedsson L, Jönsson G, Geborek P. Influence of methotrexate, TNF blockers and prednisolone on antibody responses to pneumococcal polysaccharide vaccine in patients with rheumatoid arthritis. Rheumatology (Oxford) 2006;45:106-111.
118. Nived P, Pettersson Å, Jönsson G, et al. Methotrexate reduces circulating Th17 cells and impairs plasmablast and memory B cell expansions following pneumococcal conjugate immunization in RA patients. Sci Rep 2021;11:9199.
119. Tuomanen EI, Austrian R, Masure HR. Pathogenesis of pneumococcal infection. N Engl J Med 1995;332:1280-1284.
120. Väkeväinen M, Jansen W, Saeland E, et al. Are the opsonophagocytic activities of antibodies in infant sera measured by different pneumococcal phagocytosis assays comparable? Clin Diagn Lab Immunol 2001;8:363-369.
121. Anttila M, Voutilainen M, Jäntti V, Eskola J, Käyhty H. Contribution of serotype-specific IgG concentration, IgG subclasses and relative antibody avidity to opsonophagocytic activity against Streptococcus pneumoniae. Clin Exp Immunol 1999;118:402-407.

122. Schuerman L, Wysocki J, Tejedor JC, Knuf M, Kim KH, Poolman J. Prediction of pneumococcal conjugate vaccine effectiveness against invasive pneumococcal disease using opsonophagocytic activity and antibody concentrations determined by enzyme-linked immunosorbent assay with 22F adsorption. Clin Vaccine Immunol 2011;18:2161-2167.
123. Karlsson J, Roalfe L, Hogevik H, et al. Poor Correlation between Pneumococcal IgG and IgM Titers and Opsonophagocytic Activity in Vaccinated Patients with Multiple Myeloma and Waldenstrom's Macroglobulinemia. Clin Vaccine Immunol 2016;23:379-385.
124. Migita K, Akeda Y, Akazawa M, et al. Opsonic and Antibody Responses to Pneumococcal Polysaccharide in Rheumatoid Arthritis Patients Receiving Golimumab Plus Methotrexate. Medicine (Baltimore) 2015;94:e2184.
125. Romero-Steiner S, Frasch CE, Carlone G, Fleck RA, Goldblatt D, Nahm MH. Use of opsonophagocytosis for serological evaluation of pneumococcal vaccines. Clin Vaccine Immunol 2006;13:165-169.
126. Farber DL, Yudanin NA, Restifo NP. Human memory T cells: generation, compartmentalization and homeostasis. Nat Rev Immunol 2014;14:24-35.
127. Pennock ND, White JT, Cross EW, Cheney EE, Tamburini BA, Kedl RM. T cell responses: naive to memory and everything in between. Adv Physiol Educ 2013;37:273-283.
128. Hammarlund E, Lewis MW, Hansen SG, et al. Duration of antiviral immunity after smallpox vaccination. Nat Med 2003;9:1131-1137.
129. He SWJ, van de Garde MDB, Pieren DKJ, et al. Diminished Pneumococcal-Specific CD4(+) T-Cell Response is Associated With Increased Regulatory T Cells at Older Age. Front Aging 2021;2:746295.
130. Chapman TJ, Pichichero ME, Kaur R. Comparison of pneumococcal conjugate vaccine (PCV-13) cellular immune responses after primary and booster doses of vaccine. Hum Vaccin Immunother 2020;16:3201-3207.
131. Sterrett S, Peng BJ, Burton RL, et al. Peripheral CD4 T follicular cells induced by a conjugated pneumococcal vaccine correlate with enhanced opsonophagocytic antibody responses in younger individuals. Vaccine 2020;38:1778-1786.
132. Davey Smith G, Ebrahim S, Lewis S, Hansell AL, Palmer LJ, Burton PR. Genetic epidemiology and public health: hope, hype, and future prospects. Lancet 2005;366:1484-1498.
133. Hindorff LA, Gillanders EM, Manolio TA. Genetic architecture of cancer and other complex diseases: lessons learned and future directions. Carcinogenesis 2011;32:945-954.
134. The International HapMap Project. Nature 2003;426:789-796.
135. Cano-Gamez E, Trynka G. From GWAS to Function: Using Functional Genomics to Identify the Mechanisms Underlying Complex Diseases. Front Genet 2020;11:424.
136. Kondratyev NV, Alfimova MV, Golov AK, Golimbet VE. Bench Research Informed by GWAS Results. Cells 2021;10.
137. Adan A, Alizada G, Kiraz Y, Baran Y, Nalbant A. Flow cytometry: basic principles and applications. Crit Rev Biotechnol 2017;37:163-176.
138. Leavesley SJ, Britain AL, Cichon LK, Nikolaev VO, Rich TC. Assessing FRET using spectral techniques. Cytometry A 2013;83:898-912.
139. McKinnon KM. Flow Cytometry: An Overview. Curr Protoc Immunol 2018;120:5.1.1-5.1.11.
140. Clark G, Stockinger H, Balderas R, et al. Nomenclature of CD molecules from the Tenth Human Leucocyte Differentiation Antigen Workshop. Clin Transl Immunology 2016;5:e57.

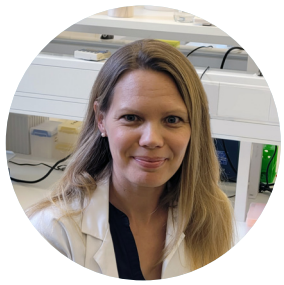
141. Wlodkowic D, Telford W, Skommer J, Darzynkiewicz Z. Apoptosis and beyond: cytometry in studies of programmed cell death. Methods Cell Biol 2011;103:55-98.
142. Cherian S, Hedley BD, Keeney M. Common flow cytometry pitfalls in diagnostic hematopathology. Cytometry B Clin Cytom 2019;96:449-463.
143. Kasinrerk W. A flow cytometric method for enumeration of lymphocyte sub-populations in sample containing lysis-resistant red blood cells. Immunol Lett 2003;86:259-264.
144. Craig FE, Ohori NP, Gorrill TS, Swerdlow SH. Flow cytometric immunophenotyping of cerebrospinal fluid specimens. Am J Clin Pathol 2011;135:22-34.
145. Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. Nat Rev Immunol 2012;12:191-200.
146. Welter D, MacArthur J, Morales J, et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res 2014;42:D1001-1006.
147. Korinfskaya S, Parameswaran S, Weirauch MT, Barski A. Runx Transcription Factors in T Cells-What Is Beyond Thymic Development? Front Immunol 2021;12:701924.
148. Watanabe K, Sugai M, Nambu Y, et al. Requirement for Runx proteins in IgA class switching acting downstream of TGF-beta 1 and retinoic acid signaling. J Immunol 2010;184:2785-2792.
149. McConnell BB, Yang VW. Mammalian Krüppel-like factors in health and diseases. Physiol Rev 2010;90:1337-1381.
150. Johnsen SA, Subramaniam M, Janknecht R, Spelsberg TC. TGFbeta inducible early gene enhances TGFbeta/Smad-dependent transcriptional responses. Oncogene 2002;21:5783-5790.
151. Kehrl JH, Thevenin C, Rieckmann P, Fauci AS. Transforming growth factor-beta suppresses human B lymphocyte Ig production by inhibiting synthesis and the switch from the membrane form to the secreted form of Ig mRNA. J Immunol 1991;146:4016-4023.
152. Bishop GA, Stunz LL, Hostager BS. TRAF3 as a Multifaceted Regulator of B Lymphocyte Survival and Activation. Front Immunol 2018;9:2161.
153. Jabara HH, Weng Y, Sannikova T, Geha RS. TRAF2 and TRAF3 independently mediate Ig class switching driven by CD40. Int Immunol 2009;21:477-488.
154. Markkanen E, Dorn J, Hübscher U. MUTYH DNA glycosylase: the rationale for removing undamaged bases from the DNA. Front Genet 2013;4:18.
155. Ohtsubo T, Nishioka K, Imaiso Y, et al. Identification of human MutY homolog (hMYH) as a repair enzyme for 2-hydroxyadenine in DNA and detection of multiple forms of hMYH located in nuclei and mitochondria. Nucleic Acids Res 2000;28:1355-1364.
156. Stratigopoulou M, van Dam TP, Guikema JEJ. Base Excision Repair in the Immune System: Small DNA Lesions With Big Consequences. Front Immunol 2020;11:1084.
157. Rölle A, Pollmann J, Ewen EM, et al. IL-12-producing monocytes and HLA-E control HCMV-driven NKG2C+ NK cell expansion. J Clin Invest 2014;124:5305-5316.
158. Gracey E, Vereecke L, McGovern D, et al. Revisiting the gut-joint axis: links between gut inflammation and spondyloarthritis. Nat Rev Rheumatol 2020;16:415-433.
159. Kenna TJ, Hanson A, Costello ME, Brown MA. Functional Genomics and Its Bench-to-Bedside Translation Pertaining to the Identified Susceptibility Alleles and Loci in Ankylosing Spondylitis. Curr Rheumatol Rep 2016;18:63.
160. Tadema H, Abdulahad WH, Lepse N, Stegeman CA, Kallenberg CG, Heeringa P. Bacterial DNA motifs trigger ANCA production in ANCA-associated vasculitis in remission. Rheumatology (Oxford) 2011;50:689-696.

161. von Borstel A, Land J, Abdulahad WH, et al. CD27(+)CD38(hi) B Cell Frequency During Remission Predicts Relapsing Disease in Granulomatosis With Polyangiitis Patients. Front Immunol 2019;10:2221.
162. Lepse N, Abdulahad WH, Rutgers A, Kallenberg CG, Stegeman CA, Heeringa P. Altered B cell balance, but unaffected B cell capacity to limit monocyte activation in anti-neutrophil cytoplasmic antibody-associated vasculitis in remission. Rheumatology (Oxford) 2014;53:1683-1692.
163. Simon Q, Pers JO, Cornec D, Le Pottier L, Mageed RA, Hillion S. In-depth characterization of CD24(high)CD38(high) transitional human B cells reveals different regulatory profiles. J Allergy Clin Immunol 2016;137:1577-1584.e1510.
164. Lee-Chang C, Top I, Zéphir H, et al. Primed status of transitional B cells associated with their presence in the cerebrospinal fluid in early phases of multiple sclerosis. Clin Immunol 2011;139:12-20.
165. Quan C, Yu H, Qiao J, et al. Impaired regulatory function and enhanced intrathecal activation of B cells in neuromyelitis optica: distinct from multiple sclerosis. Mult Scler 2013;19:289-298.
166. Carvajal Alegria G, Gazeau P, Hillion S, Daien CI, Cornec DYK. Could Lymphocyte Profiling be Useful to Diagnose Systemic Autoimmune Diseases? Clin Rev Allergy Immunol 2017;53:219-236.
167. Reddy V, Klein C, Isenberg DA, et al. Obinutuzumab induces superior B-cell cytotoxicity to rituximab in rheumatoid arthritis and systemic lupus erythematosus patient samples. Rheumatology (Oxford) 2017;56:1227-1237.
168. Appelgren D, Eriksson P, Ernerudh J, Segelmark M. Marginal-Zone B-Cells Are Main Producers of IgM in Humans, and Are Reduced in Patients With Autoimmune Vasculitis. Front Immunol 2018;9:2242.
169. Jacobi AM, Mei H, Hoyer BF, et al. HLA-DRhigh/CD27high plasmablasts indicate active disease in patients with systemic lupus erythematosus. Ann Rheum Dis 2010;69:305-308.
170. Odendahl M, Keitzer R, Wahn U, et al. Perturbations of peripheral B lymphocyte homoeostasis in children with systemic lupus erythematosus. Ann Rheum Dis 2003;62:851-858.
171. Lin W, Zhang P, Chen H, et al. Circulating plasmablasts/plasma cells: a potential biomarker for IgG4-related disease. Arthritis Res Ther 2017;19:25.
172. Stone JH, Merkel PA, Spiera R, et al. Rituximab versus cyclophosphamide for ANCA-associated vasculitis. N Engl J Med 2010;363:221-232.
173. Jenks SA, Cashman KS, Zumaquero E, et al. Distinct Effector B Cells Induced by Unregulated Toll-like Receptor 7 Contribute to Pathogenic Responses in Systemic Lupus Erythematosus. Immunity 2018;49:725-739.e726.
174. Hua C, Barnetche T, Combe B, Morel J. Effect of methotrexate, anti-tumor necrosis factor α, and rituximab on the immune response to influenza and pneumococcal vaccines in patients with rheumatoid arthritis: a systematic review and meta-analysis. Arthritis Care Res (Hoboken) 2014;66:1016-1026.
175. Subesinghe S, Bechman K, Rutherford AI, Goldblatt D, Galloway JB. A Systematic Review and Metaanalysis of Antirheumatic Drugs and Vaccine Immunogenicity in Rheumatoid Arthritis. J Rheumatol 2018;45:733-744.

176. Park JK, Lee MA, Lee EY, et al. Effect of methotrexate discontinuation on efficacy of seasonal influenza vaccination in patients with rheumatoid arthritis: a randomised clinical trial. Ann Rheum Dis 2017;76:1559-1565.
177. Park JK, Lee YJ, Shin K, et al. Impact of temporary methotrexate discontinuation for 2 weeks on immunogenicity of seasonal influenza vaccination in patients with rheumatoid arthritis: a randomised clinical trial. Ann Rheum Dis 2018;77:898-904.
178. Diks AM, Khatri I, Oosten LEM, et al. Highly Sensitive Flow Cytometry Allows Monitoring of Changes in Circulating Immune Cells in Blood After Tdap Booster Vaccination. Front Immunol 2021;12:666953.
179. Morel S, Didierlaurent A, Bourguignon P, et al. Adjuvant System AS03 containing α-tocopherol modulates innate immune response and leads to improved adaptive immunity. Vaccine 2011;29:2461-2473.
180. Sala E, Kuka M. The Suppressive Attitude of Inflammatory Monocytes in Antiviral Antibody Responses. Viral Immunol 2020;33:327-333.
181. Mitchell LA, Henderson AJ, Dow SW. Suppression of vaccine immunity by inflammatory monocytes. J Immunol 2012;189:5612-5621.
182. Nakaya HI, Hagan T, Duraisingham SS, et al. Systems Analysis of Immunity to Influenza Vaccination across Multiple Years and in Diverse Populations Reveals Shared Molecular Signatures. Immunity 2015;43:1186-1198.
183. Aristizábal B GÁ. Innate immune system. In: Anaya JM, Shoenfeld Y, Rojas-Villarraga A, Levy RA, Cervera R, eds. Autoimmunity: From Bench to Bedside. Bogota (Colombia)2013.
184. Abdgawad M, Gunnarsson L, Bengtsson AA, et al. Elevated neutrophil membrane expression of proteinase 3 is dependent upon CD177 expression. Clin Exp Immunol 2010;161:89-97.
185. Hu N, Westra J, Huitema MG, et al. Coexpression of CD177 and membrane proteinase 3 on neutrophils in antineutrophil cytoplasmic autoantibody-associated systemic vasculitis: anti-proteinase 3-mediated neutrophil activation is independent of the role of CD177-expressing neutrophils. Arthritis Rheum 2009;60:1548-1557.
186. Rarok AA, Stegeman CA, Limburg PC, Kallenberg CG. Neutrophil membrane expression of proteinase 3 (PR3) is related to relapse in PR3-ANCA-associated vasculitis. J Am Soc Nephrol 2002;13:2232-2238.
187. Schreiber A, Otto B, Ju X, et al. Membrane proteinase 3 expression in patients with Wegener's granulomatosis and in human hematopoietic stem cell-derived neutrophils. J Am Soc Nephrol 2005;16:2216-2224.

Printed by Media-Tryck, Lund 2022 NORDIC SWAN ECOLABEL 3041 0903

About the author



EVELINA ELMÉR is a consultant in Clinical Immunology and Transfusion Medicine at Skåne University Hospital in Lund, Sweden. She is a researcher at the Faculty of Medicine at Lund University. This thesis focuses primarily on the phenotypic alterations of circulating immune cells in autoimmune diseases such as ANCA-associated vasculitis and rheumatoid arthritis.

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Laboratory Medicine

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2022:137
ISBN 978-91-8021-299-1
ISSN 1652-8220