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PATTERN RECOGNITION AND INKT CELL REGULATION OF B CELL ACTIVATION

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Cover illustration: CD36 is colocalized with LC3B after autophagy induction. By Chenfei He.

Pattern recognition and iNKT cell regulation of B cell activation

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Chenfei He

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“A journey of a thousand miles begins with a single step.”

-**Lao Tzu**

ABSTRACT

The immune system protects individuals against infections but can cause disease if the response is unbalanced. Immune responses include both innate and adaptive response, and these systems are connected. As the first line of defense, innate immune responses are immediately induced upon infection. In contrast, adaptive immune responses respond to antigens in a delayed but more specific and effective. The adaptive immune responses are classified into T-independent and T-dependent responses, and B cells play a vital role in antigen presentation and antibody production in both types of immune responses. Moreover, B cells are crucial in maintaining immune tolerance, and unregulated B cells may enhance autoimmune disorders. This thesis focuses on B cell regulation in immune responses.

Paper I identifies a novel interaction of scavenger receptor CD36 with LC3B regulating autophagosome generation in B cells in adaptive immune responses. CD36-deficient B cells exhibited a significantly reduced formation of plasma cells (PCs) and altered metabolism. These changes are accompanied by the impaired formation of autophagosomes. Autophagy induction led to colocalization of CD36 with autophagosome membrane protein LC3. Mice lacking CD36 in B cells had reduced germinal center (GC) responses and autophagosomes in GC B cells *in vivo*.

Paper II shows that B cell response to apoptotic cells (ACs) relies on endosomal pattern recognition receptors. Syngeneic AC injections were used to break tolerance, and unc93b1 mutant mice that lack signaling from the TLR3, TLR7, and TLR9 receptors were investigated. Autoantibodies against Ro-52/60, La, cardiolipin, and DNA were all lower in unc93b1 mutant mice. We also observed significantly less formation of GC B cells and follicular help T (Tfh) cells in unc93b1 mutant mice than WT mice.

Paper III reveals a balance between conventional and unconventional Tfh cells direct autoreactive B cells. Coadministration of α-GalCer with ACs initiated follicular helper *i*NKT (*i*NKTfh) cell formation, which promoted short-lived GC B cells and IgG1 autoantibody production while restricted Tfh cells. We also observed that deletion of CD1d specifically in B cells limited early B cell activation, *i*NKTfh cell generation, GC B cell formation, and auto-antibody production. Moreover, endosomal TLRs were required for *i*NKTfh cell-regulated GC response.

In this thesis, we collectively evaluated the role of CD36, TLR3/7/9, and CD1d in regulating B cells in immune or autoimmune responses. It identifies critical players of CD36 in T-dependent

immune response, TLR3/7/9 in autoimmunity, and *i*NKTfh cell-mediated help to autoreactive B cells. These studies contribute to our understanding of the connection between innate and adaptive immune responses.

LIST OF SCIENTIFIC PAPERS

I. **Chenfei He**, Shan Wang, Chikai Zhou, Minghui He, Jin Wang, Marcus Ladds, Danai Lianoudaki, Saikiran K. Sedimbi, David P. Lane, Lisa S. Westerberg, Shuijie Li*, Mikael C.I. Karlsson*.
CD36 and LC3B initiated autophagy in B cells regulates the humoral immune response. *Autophagy 2021:1-15*

II. Manasa G. Garimella, **Chenfei He**, Guangchun Chen, Quan-Zhen Li, Xin Huang, Mikael C. I. Karlsson
The B cell response to both protein and nucleic acid antigens displayed on apoptotic cells is dependent on endosomal pattern recognition receptors. *Journal of Autoimmunity 117 (2020): 102582*

III. **Chenfei He**, Shan Wang, Shengduo Pei, Facundo D. Batista, Mikael C.I. Karlsson
The balance between conventional and unconventional follicular helper T cells direct autoreactive B cells. *Manuscript*

LIST OF RELATED PUBLICATIONS NOT INCLUDED IN THIS THESIS

I. Yu Gao, Ruining Liu, **Chenfei He**, Juan Basile, Mattias Vesterlund, Marie Wahren-Herlenius, Alexander Espinoza, Cassandra Hokka-Zakrisson, Fahad Al Zadjali, Akihiko Yoshimura, Mikael C.I. Karlsson, Berit Carow, Martin Rottenberg
SOCS3 expression by thymic stromal cells is required for normal T cell development *Frontiers Immunology, 2021, 12: 668.*

II. Yesid Estupiñán, Thibault Bouderlique, **Chenfei He**, Anna Berglöf, Dhanu Gupta, Osama Saher, Miguel Ángel Daza Cruz, Lucia Peña-Perez, Liang Yu, Rula Zain, Mikael CI Karlsson, Robert Månsson, CI Edvard Smith
Novel mouse model resistant to irreversible BTK inhibitors: a tool identifying new therapeutic targets and side effects *Blood advances, 2020, 4(11): 2439-2450.*

CONTENTS

LIST OF ABBREVIATIONS

AC	Apoptotic cell
ADCC	Antibody-dependent cell cytotoxicity
AID	Activation-induced cytidine deaminase
APC	Antigen presentation cell
ATG5	Autophagy-related gene 5
ATP	Adenosine triphosphate
BCR	B cell receptor
CD	Cluster of differentiation
CD40L	CD40 ligand
CPG	Unmethylated cytosine-guanosine
CQ	Chloroquine
CXCR	CXC receptor
DC	Dendritic cell
Fc	Fragment, crystalizable
FDC	Follicular dendritic cell
FOB	Follicular B cell
GC	Germinal center
i.p.	Intraperitoneally
i.v.	Intravenously
IFN-γ	Interferon-gamma
Ig	Immunoglobulin
IL	Interleukin
*i*NKT	Invariant nature killer T
*i*NKTfh	Follicular helper invariant nature killer T
LPS	Lipopolysaccharide
MAP1LC3/LC3	Microtubule-associated protein 1 light chain 3
MFI	Mean fluorescence intensity
MHC	Major-histocompatibility-complex
MZB	Marginal zone B cell
NP-CGG	4-hydroxy-3-nitrophenylacetyl-chicken gamma globulin
OCR	Oxygen consumption rate
oxLDL	Oxidized low-density lipoprotein

PC	Plasma cell
Rapa	Rapamycin
SLE	Systemic lupus erythematosus
SQSTM1/p62	Sequestosome 1
SRBC	Sheep red blood cell
TCR	T cell receptor
Tfh cell	Follicular helper T cell
TLR	Toll-like receptor
α-GalCer	α-GalactosylCeramide

1. INTRODUCTION

1.1 Overview of the immune system

The immune system is responsible for preventing infection and keeping a balance in our bodies. To recognize and eliminate a diversity of microbes, humans have evolved an intricate network of cells that communicate and maintain immunity. The immune system needs to interact with others, such as the metabolic, nervous, and endocrine systems. Another requirement of the immune system is to maintain tolerance to self-proteins and nuclear acids. The absence of self-tolerance will cause the immune system to attack our bodies leading to autoimmune diseases.

From a historical perspective, the study of the immune system is driven by the human desire to survive infectious diseases. The earliest described observation of immunity was in 430 BC. Thucydides, the great historian of the Peloponnesian war, wrote that people who had survived through the plague could take care of the plague patients without contracting the disease. In the fifteenth century, the Chinese and Turks attempted to induce immunity. They used the dried crusts from the smallpox pustules to insert into minor cuts in the skin to prevent this disease[1]. In 1798, English physician Edward Jenner discovered that milkmaids infected with the mild disease cowpox had immunity to severe smallpox. The inoculating with cowpox that protected against smallpox spread quickly through Europe. Pasteur found microbes in this infectious disease and extended his discovery to other conditions, showing that attenuated microbes can be used as a vaccine to protect from infection. Subsequently, in 1885, Pasteur successfully developed the first attenuated vaccine for the rabies virus[2].

Although Pasteur showed that vaccination was effective, he did not understand the underlying mechanisms. In 1890, the study showed that serum from vaccinated animals could protect others[3]. Another early observation done at the same time was when Elie Metchnikoff observed that phagocytes ingested microorganisms and other foreign material. In 1930, it was shown that a component from serum from an immunized individual named gamma globulin was required for immune activation by Elvin Kabat[4]. The immunologic events mediated by immunoglobulin were subsequently named humoral immunity. The immunity induced by specific cells was then called cell-mediated immunity. During an immune response, two systems called innate and adaptive immunity are interconnected and regulate each other. Innate immunity is the first defense line to prevent infections and quickly eliminates foreign antigens. The adaptive immune response involves B and T lymphocytes with increased specificity,

including several epitopes on an individual antigen. It takes 5 or 6 days after the initial exposure to develop an adaptive immune response against a pathogen. The signals produced by the innate immune cause inflammation required for the adaptive immune response. An example of this is that adjuvants are needed for vaccination against a specific antigen.

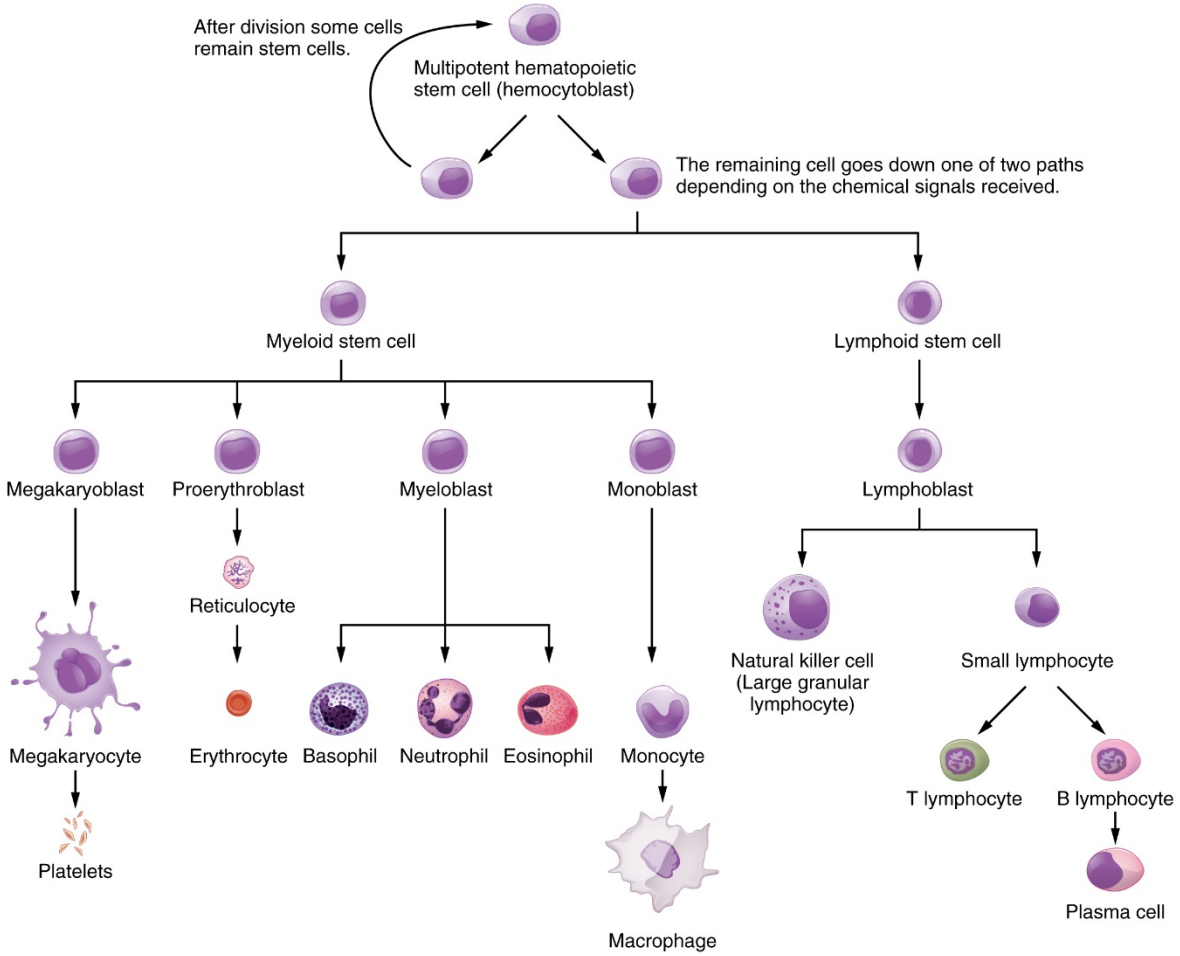

Figure 1. Illustration of the development of immune cells. All immune cells originate from hematopoietic stem cells. Taken from #6.

1.2 Lymphoid organs

All blood cells develop from the hematopoietic stem cells (HSC) (**Figure 1**)[5, 6]. Hematopoiesis starts during embryonic development when precursor cells in the yolk sac differentiate into nucleated erythroid cells. Further, mature HSC is found in multiple tissues during embryogenesis, including the yolk sac, placenta, and fetal liver. These HSC can differentiate into both lymphoid cells and myeloid cells. The central lymphoid organ is where immune cells develop from HSCs through the myeloid and lymphoid pathways. The peripheral lymphoid organ is where naïve lymphocytes initiate the immune response. The central lymphoid organs are composed of bone marrow and thymus. The peripheral lymphoid organs

comprise the spleen, lymph node, tonsils, Peyer's patches, and mucosa-associated lymphoid tissue, where immune cells encounter antigens and induce the immune response.

As the largest lymphoid organ, the spleen is a critical player in triggering immune responses against blood-borne antigens. Anatomically, the spleen can be divided into white and red pulp. Lymphoid follicles together form the white pulp. A follicle is composed of surrounded a B cell zone and a T cell zone in the center required for the GC response against protein antigens. The marginal zone is localized on the edge of the white pulp, which connects the blood circulation. Marginal zones serve a critical role in capturing blood-borne microbes by phagocytes there[7]. Thus, the marginal zone contributes to initiating an adaptive immune response. The red pulp is on the outside of the white pulp marginal zones. Macrophages and DCs in the red pulp search for pathogens and clear up the damaged red blood cells.

1.3 The innate immune system

The innate immune system provides early and immediate protection against microbes and causes inflammation. Natural immunity displays in individuals even in a healthy status participate in the block and eliminate microbes from entering the host. The innate immune system includes physical barriers, chemical barriers, and cellular responses against infection. Physical barriers including skin, mucosal, and glandular tissue as the first defense line. Our defense against infections also includes chemical barriers have anti-microbial substances, acid PH, complement proteins, and substances originating from granulocytic innate immune cells. The innate immune cells are mainly made up of myeloid cells such as DCs, monocytes, macrophages, granulocytes, and lymphoid cells, including natural killer (NK) cells and innate lymphoid cells (ILC). Myeloid cells can function as antigen-presenting cells during being stimulated to eliminate extracellular pathogens through phagocytosis or pinocytosis. Other specific receptors elicit the release of proteins and anti-microbial substances and cause swelling and other physiological changes that are part of inflammation. Pattern recognition receptors play essential roles in detecting pathogens and activating innate immune responses through recognition conserved through evolution. They appear on the cell surface but also intracellularly. Thus, evolution has provided us with pattern recognition receptors recognizing molecular patterns on pathogens that induce cell signaling activation. Besides, damage-associated molecular patterns released by cells and tissue can be identified by these receptors.

Examples of receptors on the cell surface are Toll-like receptors (TLRs), scavenger receptors, c-type lectin receptors, complement receptors, and intracellular receptors, including NOD-like

receptors and retinoic acid-inducible gene (RIG)-I-like receptors[8, 9]. TLRs were the first discovered pattern recognition receptors in the 1980s, and they are characterized by the shared protein structure of leucine-rich repeats. So far, 13 TLRs have been identified in humans and mice. Some TLRs on the cell surface recognize components on pathogens, while some endosomal TLRs recognize elements released during the degradation of microbes or as a consequence of necrosis[10]. Their cellular location enables them to respond optimally to recognize microbial ligands and self-antigens. The innate responses are sufficient to eliminate or at least control infections, and insects rely only on this system for their defense [11, 12]. For vertebrates, the natural and inflammatory response is often not enough to clear infections, and then the adaptive immune response comes to play and can also induce memory.

1.3.1 Scavenger receptors

Scavenger receptors are pattern recognition receptors displayed mainly on the cell surface. In 1979, Drs. Brown and Golstein found that scavenger receptors could bind with modified low-density lipoprotein, which defines the family. Many other ligands have been discovered since then, and the ligand-binding domain is a conserved cysteine-rich region. The scavenger receptor family includes lectin-like oxidized low-density lipoprotein receptor (LOX)-1, MACRO, and CD68, scavenger receptor class A (SRA), scavenger receptor class B (SRA), and others[13-16]. Scavenger receptors can recognize molecular patterns displayed on pathogens or infected cells such as lipoproteins, cholesterol ester, phospholipids. They can also identify modified self-antigens, such as modified low-density lipoprotein and apoptotic cells. Scavenger receptors initiate innate immune responses, especially phagocytosis, to eliminate foreign antigens during the infection[17, 18].

The CD36 scavenger receptor belongs to the class B family and was first described by Paul A. Grimaldi in 1993[19]. CD36 has 472-amino acids and is a heavily glycosylated transmembrane protein. The extracellular region has a phosphorylation site at Thr92. Phosphorylation of CD36 is critical for mediating adherence with other cells and binding ligands[20-22] and the interaction with lipid rafts to increase long-chain fatty acid uptake[23, 24]. CD36 is expressed by many cell types, including monocytes, lymphocytes, cardiac muscle cells, skeletal muscle cells, and adipocytes. The binding ligands include apoptotic cells, native or modified lipoproteins, glycated proteins, thrombospondin-1and long-chain fatty acids[25]. CD36 contributes to recognizing pathogens, damaged tissues, and opsonin-independent pathogen internalization and phagocytosis. CD36 can mediate apoptotic cells uptaken by DCs and play a

role in cross-present antigens to $CD8^+$ T cells. CD36 is highly expressed by marginal zone B cells (MZBs) and low on FOBs on lymphocytes, and it has been shown to mediate T-independent response to heat-killed Streptococcus pneumoniae[26]. CD36 can transport long-chain fatty acid and oxLDL in adipocytes, enterocytes, muscle cells, and hepatocytes. Besides the immune system, CD36 expression in muscle tissue is associated with oxidative potential regulated by insulin[27, 28]. An essential function of CD36 on the cell surface is to enhance fatty acid uptake. In mice lacking CD36, the fatty acid uptake and binding of oxLDL were lower by peritoneal macrophages than WT mice[29]. CD36 serves a role in autophagy as well. Atg5-deficient DCs have elevated CD36 expression and lipid accumulation[30]. The deletion of CD36 down-regulates CD5L-mediated autophagy and cytokine production in macrophages [31]. CD36 is also responsible for taking up long-chain fatty acid and oxLDL in adipocytes, enterocytes, muscle cells, and hepatocytes[32, 33].

1.3.2 Toll-like receptors

The TLRs were first discovered in drosophila and essential for dorsoventral polarity in the embryonic stage[34, 35]. They are displayed on antigen presentation cells, including B cells,

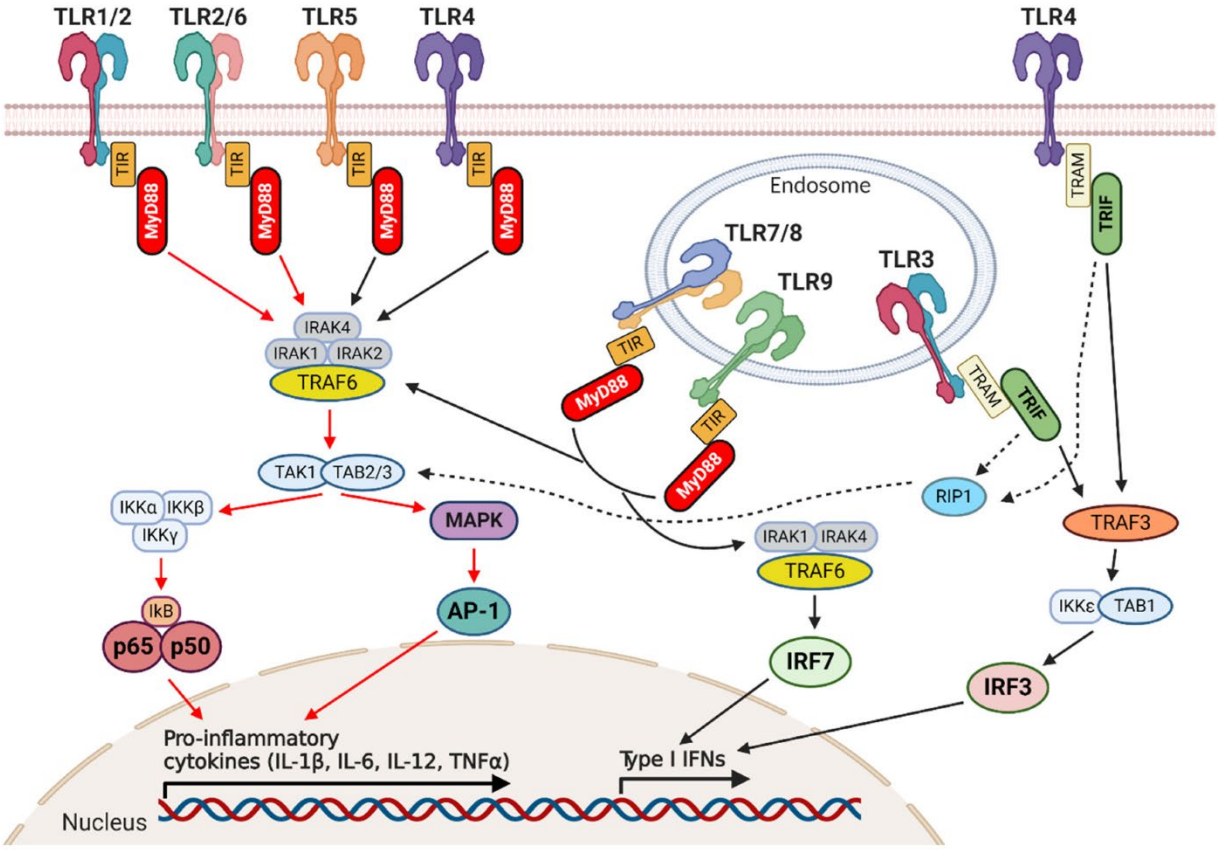

Figure 2. Toll-like receptor (TLR) signaling pathways. The figure exhibits the location and signaling pathway of TLRs. Taken from #38.

DCs, and macrophages. The TLRs include TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10 expressed in both humans and mice, whereas TLR11, TLR12, and TLR13 are only detected in mice (**Figure 2**)[36-38]. They are classified into subfamilies based on the similarity of amino acid sequences.

As pattern recognition receptors in innate immune response, the TLRs bind with structurally conserved molecules derived from pathogens and lead to the activation of costimulatory signals and secretion of inflammatory cytokines. TLR1-6 and TLR10-12 are expressed on the cell surface and recognize microbes-derived molecules[39, 40]. Specifically, TLR4 on the surface is responsible for identifying lipopolysaccharide (LPS) in Gram-negative bacteria[41]. TLR3, TLR7, TLR8, TLR9, and TLR13 in mice are expressed in the endosomes, multivesicular bodies, endoplasmic reticulum, and lysosomes. They are responsible for identifying nucleic acids from both pathogen and damaged cells. TLR3 recognizes dsRNA and synthetic derivatives like polyinosinic-polycytidylic acid (poly I:C)[42]. TLR7 is specific for ssRNA analog-like resiquimod (R848)[43]. TLR9 is responsible for detecting bacterial DNA and synthetic analog CpG[44].

The signaling pathway from TLRs is similar to the mammalian IL-1 pathway showing a connection between these receptors and cytokines necessary for inflammatory responses[45]. Except for TLR3, all other TLRs rely on the MyD88 signaling pathway. Ligand and leucine-rich repeats in TLRs ectodomain trigger signaling through the alteration in the conformation and the association of the Toll/interleukin-1 receptor domains[46]. The connection between the Toll-interleukin receptor (TIR) domains and adaptor molecules is required for signaling. Mutations in MyD88 may cause selective influence on receptors [47], while certain MyD88 TIR domains are responsible for the recruitment and activation of downstream signaling[48, 49]. However, TLR3 signal through the adaptor adapter-inducing interferon-β (TRIF)[50]. TRIF connects with TNF receptor-associated factor 3 (TRAF 3) and TRAF6 and receptor-interacting proteins 1 and 3. TRAF3 binds TANK-binding kinase 1 (TBK1) to the TRIF-dependent pathway, which is associated with the inhibitor of nuclear factor kappa-B kinase 3 (IKK3), phosphorylates, and activates interferon regulatory factor 3 (IRF3), causing IFNβ production[51, 52]. TLR4 signals through the same TRIF pathway, but it needs the TRIF-related adaptor molecule under most situations.

TLRs detecting host DNA can be involved in self-tolerance, and the interaction of self-DNA with endosomal TLRs is a possible trigger for autoimmune diseases. It has been shown that stimulation of intracellular TLRs, especially TLR7 and TLR9, promotes autoantibodies against

nuclear acid[53]. Moreover, the combination of activation of the BCR and endosomal TLRs enhances autoantibody production. Nucleic acid-sensing in other immune cells like DC can enhance autoreactive B cells[54]. After transcription, TLRs 3, 7, and 9 are coupled to membrane protein Unc93b1 to be transported from the endoplasmic reticulum to the endosomes to process and display antigens. Mice lacking Unc93b1 fail to initiate sufficient immune response preventing against auto-antigens[55, 56].

1.3.3 Innate immune cells

Innate myeloid cells include granulocytes, monocytes, and macrophages. Except for myeloid cells, lymphocytes such as NK cells and ILCs contribute to the natural immune response.

1.3.3.1 Granulocytes

Granulocytes respond rapidly during an innate immune response, and the cell types include neutrophils, eosinophils, basophils, and mast cells. All granulocytes have multilobed nuclei and are relatively large cells that distinguish them from lymphocytes. Granulocytes are also distinguishable by cytoplasmic granules that stain differently depending on content. These granules contain an extensive array of proteins and serve a vital role in the innate immune response as drivers of inflammation. Some enhance the remodeling of tissue in infection, and some directly eliminate pathogens.

Neutrophils constitute about 50% to 70% of peripheral white blood cells and are the most numerous granulocytes. During infection, innate immune cells generate inflammatory molecules to promote the development of neutrophils from the bone marrow. The mature neutrophil migrates to the infection site and extravasates to the tissue. Here, they phagocytose bacteria and secrete various anti-microbial and tissue-remodeling proteins. Neutrophils can also regulate B cell responses by developing into B helper Neutrophils, and it has been shown that these can support a B cell response by the production of BAFF[57, 58]. Eosinophils can be stained a brilliant pink in standard H&E staining due to specific granules. Functionally, eosinophils are essential in defending against parasites, asthma, and allergy. They can also support PCs survival by producing the TNF family member APRIL. Basophils have large granules that stain blue in standard H&E staining. Basophils are a critical player in response to parasites, particularly helminths. In allergy, circulating antibody and antigen complex induces histamine release from their granules, which results in increased smooth muscle activity, enhanced blood vessel permeability, and allowing immune cells to access the infection site.

Mast cells also play a vital role in defending against parasitic worms and cause allergies. All granulocytes can release cytokines that regulate other immune cells and initiate the adaptive immune response.

1.3.3.2 Dendritic cells

Dendritic cells (DCs) develop from monocytes that make up 2%-12% of leukocytes in the blood. Monocytes can differentiate into various mature cell types after migrating into specific tissues, where the two main ones are DC and macrophages. The close ontogeny between DC and macrophages is exemplified by Langerhans cells that reside in the skin. They were thought to be DC, but the finding that they develop from an embryonically derived macrophage stem cell in the tissue changed their classification[59]. The importance of this cell type for the immune response was highlighted when Ralph Steinman was granted the Nobel prize in 2011 to discover DC in the mid-1970s.

DCs functions as antigen-presenting cells and messengers between innate and adaptive immunity. Once they have encountered antigens, they take up antigens by pinocytosis and display them to major histocompatibility complex (MHC). Activated DCs can migrate and enhance antigen presentation and expression of costimulatory molecules. The mature DCs move to lymphoid organs and participate in antigen presentation. DCs can take up, process, and display extracellular antigens on MHC I and present to $CD8^+$ T cells, called cross-presentation. It is critical in an immune response against viruses and tumors[60, 61]. Conventional DCs can be classified into $CD8\alpha^+$ cDC1 and $CD11b^+$ cDC2[62].

1.3.3.3 Macrophages

Some monocytes migrate into tissues in response to infection and differentiate into macrophages. The pro-inflammatory macrophages are essential in phagocytes and contribute to an effective innate immune response. By being activated by pathogens or tissue damage, they undergo phenotypical and functional changes and participate in the clearance of pathogens. It was recently shown that macrophages could also be originated from stem cells in the tissue that arise during embryonic development[63]. They are a very plastic population of cells, and cues from the tissue will determine their polarization and function.

Recent studies found that surprisingly, most tissue-resident macrophages arise from embryonic cells instead of circulating monocytes. Tissue-resident macrophages include microglia in the brain, Kupffer cells in the liver, and alveolar macrophages in the lung. They keep the ability to

self-renew. They co-exist with circulating macrophages and share their function as professional antigen-presenting cells. Macrophages are specialists for pattern recognition, and they also have Fc-receptors that can recognize immune complexes. Once a pathogen is coated with a specific antibody during an immune response, macrophages will bind to the immune complex, enhancing phagocytosis.

1.3.3.4 Natural killer cells

Nature killer cells were firstly discovered by Herberman et al. at the University of Pittsburgh and Rolf Kiessling, Hans Wigzell, and Eva Klein at Karolinska Institutet and in1975[64-67]. NK cells represent about 5%-10% of lymphocytes in blood and are defined by the expression of several surface markers, including NK1.1. They are part of the ILC family, belong to cytotoxic ILCs, distinct from helper-like ILCs like ILC1, ILC2, and ILC3[68]. They regulate the immune system via the production of cytokines and induce cytotoxicity to provide the first line of defense against the pathogens in the mucosal tissue and skin. They are also crucial for detecting tumor cells which is how they were discovered.

NK cells release cytotoxic granules into extracellular space when encountering infected cells in infection. Besides this, stimulated NK cells can synthesize and produce the cytokine interferon-γ (IFN-γ) to active macrophages[69]. Activated macrophages are more efficient in phagocyting microbes and secrete IL-12, IL-15, and type I IFN to enhance the ability of NK cells. Moreover, cytokine IL-15 from macrophages is vital in NK cell development, and IL-12 promotes the killing function[70, 71]. Collectively, NK cells and macrophages work together to prevent infection, especially for intracellular infection.

NK cells kill infected cells and tumor cells via two distinct strategies. The first strategy is to attack cells that lack self-MHC class I molecules[72]. There is often downregulating MHC class I in the affected cells in tumor cells and infections induced by the virus. On the surface of NK cells, there are many receptors for self-MHC class I that limit the killing ability of NK cells. However, when NK cells encounter cells lacking MHC class I, the inhibiting receptors are no longer engaged, and NK can release their cytotoxic granule to kill the target cell[73, 74]. Since ligands of NKp46, NPG2D, NKp44, DNAM are highly expressed by infected and malignant cells, NK cells enable to mediate killing by specific ligation[75]. NK cells also express FcgRIII, which can bind to antibody complexes. It links the immune complex of antibodies and microbes to the cell and promotes killing. Once antibodies bridge NK cells with infected cells, the NK

cells release their granules and induce cell death. This process is called antibody-dependent cell cytotoxicity (ADCC).

1.4 The adaptive immune system

The adaptive immune system has an essential role in eliminating pathogens. Antigen-specific receptors on B cell and T cell are required for adaptive immune responses and make up the unique specificity. B cells and T cells are different in recognizing antigens. The BCRs are membrane-bound antibodies that bind 3D structures and can have an affinity for many types of molecules, such as proteins, lipids, nucleic acids, polysaccharides. B cell-regulated adaptive immune response protects against various microbes, soluble antigens, and cellular debris. T cell receptors are more restricted and recognize peptides from protein antigens bound to MHC on antigen presentation cells. Therefore, different antigens and epitopes on pathogens can be identified by B cells or T cells. Upon the antigen recognition, both B cell and T cells will be activated and differentiated into effector cells. Activated B cells can differentiate into GC B cells, PCs, or memory B cells. B cells can also produce cytokines regulating the immune response during their activation. During activation, different subsets of helper T cells derived from $CD4^+$ T cells will be generated to control the adaptive immune response. The lymphoid blood cell lineage is broadly subdivided into populations based on phenotype and function. It includes T lymphocytes, B lymphocytes, unconventional T cells such as *i*NKT cells and type II NKT cells, and NK cells.

1.4.1 T lymphocytes

T lymphocytes acquire their name from their specific migration to the *thymus,* which is needed for their maturation. T lymphocytes originate from common lymphoid progenitors (CLP) and are essential in adaptive immunity. T cell receptor is the critical receptor used for their function and activation. In antigen presentation cells, MHC molecules form complexes with internalized proteins from the cell surface and present them to browsing T cells. There are two versions of MHC molecules called MHC I and II. MHC I is expressed by all nucleated cells, while MHC class II is only represented by antigen presentation cells. T cells can be classified into $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells based on the surface marker expression. Helper T cells recognize antigens displayed on MHC class II, whereas CD8 T cells identify antigens on MHC class I. DCs has the unique capacity to perform cross-presentation, which can present exogenous antigens on MHC class I to initiate $CD8^+$ T cells.

Upon the interaction of the MHC II-peptide complex, naïve $CD4^+$ T cells become activated, expand, and divide into effector T-cells[76, 77]. Effector T-cells include helper T type 1 (Th1), Th2, Th17, regulatory T cells (Treg), and Tfh cells. They have different functions in the immune response. Helper T type 1 (Th1) cells are responsible for the immune response against ingested pathogens, while Th2 cells regulate our responses to parasite infections[78, 79]. Th17 cells contribute to the immune response against extracellular bacteria and fungi and regulate the intestine's immunity. Each helper T cells secrete different cytokines that regulate the stimulation of B cell subsets, macrophages, DCs involved in the response. Another essential type of effector T cell is the Tregs that suppress the immune response mediated by helper T cells once microbes are eliminated. Tregs are characterized by the upregulation of CD4, CD25, and the transcription factor FoxP3 and regulate immune response to pathogens and stop reactions to self-antigens[80, 81]. Tfh cells provide help to activated B cells in GC response. They are essential in forming GC and providing signals to select the high affinity maturated and class-switched GC B cells and produce high-efficiency antibodies[82]. Cytokine IL-6 is required for switching from activated T cell to Tfh. Together with signaling from TCR and costimulatory molecules, these T cells will be induced the expression transcription factor Bcl-6 and become Tfh cells[83]. Tfh cells are also defined by their excrete of IL-21 contributing to B cell differentiation.

Naïve $CD8^+$ T cells need to be activated to turn into cytotoxic T cells with the ability to kill other cells. It happens after interacting with MHC I peptide-complex and co-stimulation molecules on APCs. The cytotoxic T cells play an essential role in eliminating infected or altered cells. These cells include virus-infected cells, tumor cells, and cells recognized as foreign due to tissue transplantation. Although $CD4^+$ T cells will not be directly engaged in the killing process, the provision of help from $CD4^+$ T cells is required for the proliferation and differentiation of naïve $CD8^+$ T cells.

1.4.2 B lymphocytes

In 1965, Robert Good and Max Cooper first found that cells from the Bursa Fabricius are required to produce antibodies. The B lymphocytes derive their name from these studies[84-86]. B lymphocytes are distinguished from other cells by expressing B cell receptors, membrane-bound immunoglobulin molecule. B cell development starts at the bone marrow, just like other immune cells with the HSC (**Figure 3**).

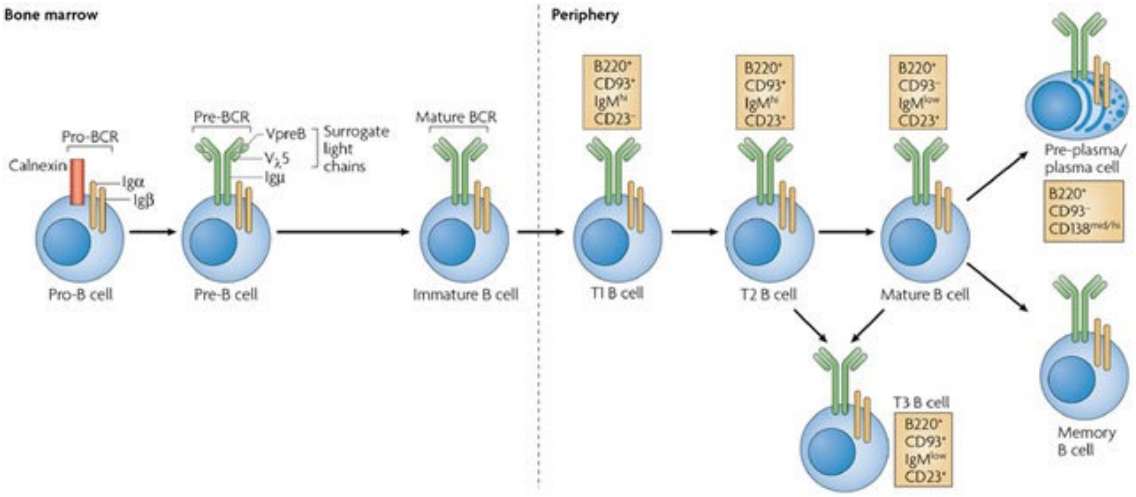

Figure 3. B cell development. The rearrangements at the gene segments of BCR lead to the formation of pre-B-cell receptor, and is followed by a mature BCR that has antigen binding ability. After selection, non-self-reactive cells leave out the bone marrow, develop into transitional B cells, and become follicular B cells or marginal-zone B cells. Under antigen activation, B cells differentiate into PCs or memory B cells. Taken from #86.

In the immune response, antigen-specific responses largely depending on diverse receptor repertoires from which clones are selected to expand. Unlike pattern recognition receptors, BCR is recombined from separate gene segments in their correspondent gene loci, called variable diversity joining (VDJ) recombination. The immense diversity of genetic sequence results in the ability of BCRs to bind 10^{13}-10^{18} different antigens[87-90]. V(D)J recombination occurs in the development of B in the bone marrow and thymus. The BCRs are composed of the subunits of receptors: Ig heavy and light chains; and α and β chains used for signaling. Ig heavy chains of BCR include VDJ segments and light chains are made up of VJ segments. The recombination of VDJ segments is regulated by enzymes containing recombination activating genes (RAG) 1 and 2, Artemi, and terminal deoxynucleotidyl transferase (TdT). Damaged DNA will be repaired by ligases, generating a recombined V-J or V-D-J exon with the help of RAG 1-2 and Artemis. TdT catalyzes the random nucleotides[91, 92]. Developing B cells create a diversity of repertoire receptors recognizing a wide variety of antigens.[93]. Self-reactive B cells are eliminated at bone marrow development stages to keep self-tolerance. This central tolerance also includes receptor revision where B cells can avoid self-reactivity by changing the light chain of the antibody. The exact mechanism for central tolerance for B cells is unknown and not as strict as T cell education in the thymus. After B cells leave the bone marrow, peripheral tolerance is mainly regulated by interactions with T cells, including Treg subsets.

Upon receiving signals from the cell-surface receptors and adhesion molecules, B cells will be in the process of differentiation and proliferation, which promote their movement within the

bone marrow environment and form the immature B cells. Immature B cells leave the bone marrow and continue their further differentiation in second lymphoid organs. Besides antibody production, B cells play a vital role in cytokine production, antigen presentation, and immune memory. The predominantly function of mature B cells is to detect pathogens and differentiate them into PCs to produce antibodies to protect our body.

1.4.2.1 B cell subsets

Once immature transitional 1 (T1) B cells leave the bone marrow, they migrate into the secondary lymphoid organs and become T2 B cells. T2 B cells phenotypically differ from T1 B cells by IgD, CD21, CD23, and BAFF-receptor expression[94]. T2 B cells will turn into B-2 B cells and become follicular B cells (FOBs) or MZBs[95, 96].

FOBs in follicles are the predominant B cell subsets in the spleen and participate in T-dependent immune response[97]. Mature FOBs are characterized by high IgD and CD23 and intermediate expression of IgM and CD21. FOBs recirculate between lymphoid organs and blood to search for their antigen displayed by FDCs. They respond to encountered T-dependent antigens by BCR uptaking and presentation. As a result, B cells will undergo proliferation and differentiation during the immune response, including class-switching and differentiation to become PCs and memory cells[95]. Memory cells continue to recirculate and induce antibody response when they re-encounter their antigen.

MZB cells get their name from their localization in the marginal zone at the edge of the white pulp. Like B-1 cells, MZB cells are capable of self-renew in the periphery and long-lived cells. They are characterized by the expression of CD21, IgM, CD36, CD9, CD1d, and relatively lower expression of CD23 and IgD[95, 96, 98]. CD21 functions as a receptor for the shuttling of antigens that have been opsonized to follicles for deposition on FDCs. The high expression of IgM and CD36 makes MZBs easier to activate and respond to antigens. MZB cells express phospholipid receptors and adhesion molecules needed to hold them in the marginal zone, and if these are blocked, MZBs go to the circulation[99, 100]. Also, the interaction between MZBs and marginal zone macrophages is essential for maintaining the MZ structure. MZBs can travel between the marginal zone and follicular to transport captured antigens into the follicles by accumulating antigens on FDCs [99, 101]. MZBs can also respond immediately to T-independent antigens stimulation and become PCs[102].

B-1 B cells are derived from the fetal liver, and most of them are located in peritoneal or pleural cavities in mice. There are B1 B cells found in spleens, but they only take up a small fraction

(about 2%) of the splenic B cells. B-1 B cells can be classified into B-1a ($CD19^{hi}CD5^{hi}$) and B-1b cells ($CD19^{hi}CD5^{lo}CD11b^{hi}$), based on the expression of the CD5 and CD11b[95, 103]. The antibody repertoire of B-1a cells is selected to recognize lipid and polysaccharide antigens from both hosts and microbes. Although helper T cells can enhance antibody affinity by somatic hypermutation, B1-a B cells can produce antibodies in the absence of help from T cells. B1-a B cells perform a role that bridges innate and adaptive immunity to make antibodies. B-1 B cells differ from B-2 B cells based on phenotypes and function.

Regulatory B cells (Bregs) are a subset of B cells contributing to immune suppression. Bregs include $CD5^+$ B1a B cells, MZBs, T2-Mprecursor, and $CD1d^{high}CD5^+$ B cells[104-106]. The suppression mechanism of Bregs is through the secretion of anti-inflammatory cytokine IL-10. Naïve B cells have no detectable IL-10 secretion, while stimulations of BCR can induce the formation of Bregs, TLR, or CD40[107, 108]. It is also known that cytokines, such as IFN-γ, IL-6, IL-21, IL-33, and IL-1β, are required for their activation[109-112]. The regulatory function of Bregs was demonstrated in various models.

1.4.2.2 B cell activation

B cell subsets respond to both non-protein and protein antigens. Antigens can be classified as T-independent and T-dependent according to T cell help requirement. Nonprotein antigens initiate immune response primarily through T-independent responses. T-independent antigens can be divided into type I (e.g., lipopolysaccharide, bacterial DNA, CPG) and type II (for example, dextran, ficoll). T-independent type I response involves TLR but not BCR signaling. In contrast, T-independent type II response is mediated by the crosslinking of multiple BCRs and require signaling through Bruton's tyrosine kinase (Btk). MZB responds quickly to nonprotein antigens in spleens, while B-1 cells in the peritoneum and mucosal tissues. The B1 cells and MZBs are often considered innate-like B cells that express B cell receptors with restricted diversity and low affinity against microbes. At the same time, they can rapidly differentiate into short-lived memory cells and IgM^+ PCs in the extrafollicular region[113].

A critical step in the T-dependent response is when protein antigens are internalized by B cells via the BCR and presented on MHC II[114, 115]. When TCR recognizes the MHC II displayed epitopes, the CD3 and ζ chains transmit initiated signals. However, T cell activation requires co-stimulation of B cells as secondary signals[116-118]. B cells are activated by several signaling pathways[113]. Firstly, antigen-induced clustering of B cell receptors triggers signals and transduce activation via immunoreceptor tyrosine-based activation motifs. The phosphor-

tyrosine recruits Syk and downstream signaling cascades[119, 120]. B cells are also stimulated by other co-receptors such as CD19, CD21, and CD81 and pattern recognition receptors[120-122]. CD21 is a receptor for C3b in the complement system that provides signals for the activation of opsonized antigens[121]. Immune response against T-dependent antigens will induce a GC response in follicles at the border to the T cell zone. FOBs and Tfh cells are the predominant cells recruited to this reaction. The same antigen-activated FOBs and made them migrate close to the T cells zone. For directing migration, activated T cells downregulate the level of chemokine receptor CCR7 on the surface. The expression of CXCR5 on cells in the follicle attracts them to B-cell zones[123, 124]. As has been mentioned, T cells express costimulatory molecules and secrete cytokines to help B cells[125, 126]. The best-characterized costimulatory molecule for T cells is two associated proteins CD80 and CD86 (named B7.1 and B7.2), recognized by protein CD28 on T cells[113, 116]. A co-receptor called ICOS (inducible costimulator) is connected to CD28 and is responsible for Tfh cell development in GC reaction[127]. Another important costimulatory molecule on T cells is the CD40 ligand, which binds to CD40 on B cells. After being fully matured by interacting with B cells, Tfh cells are characterized by expression of CXCR5, PD-1, CD40L, SAP, ICOS, Bcl-6, and secretion of IL-21[125, 126].

T-B cell interaction initiation results in GC formation with separated dark and light zones based on the histological appearance[128]. The combination of expression of CD83 or CD86 with chemokine receptor CXCR4 defines the light zone and dark zone GC B cells[129]. DZ B cells are characterized by $CD83^{low}CXCR4^{high}$ or $CD86^{low}CXCR4^{high}$, whereas LZ B cells are $CD83^{high}CXCR4^{low}$ or $CD86^{high}CXCR4^{low}$ [129, 130]. B cell expansion is the most prominent feature of the dark zone that gets its name due to the dense cellularity of proliferating cells. The light zone is occupied by B cells undergoing clonal selection by interacting with Tfh cells (**Figure 4**)[93, 131]. Somatic hypermutations result in diverse BCRs, and the best clones are selected by Tfh cells[132]. Activation-induced deaminase (AID) frequently converts uracils to thymidines in the Ig variable region and induces the mutations[133]. The class-switching occurs in the Ig heavy chain constant region and is also driven by AID. Activated B cells will differentiate into effector cells such as PCs and memory B cells. PCs continue to produce high-affinity antibodies to eliminate the pathogen[134-136]. Memory B cells are ready for responding if they re-encounter the same antigen. And some of the memories of T-dependent antigens can be sustained for many years or even a lifetime.

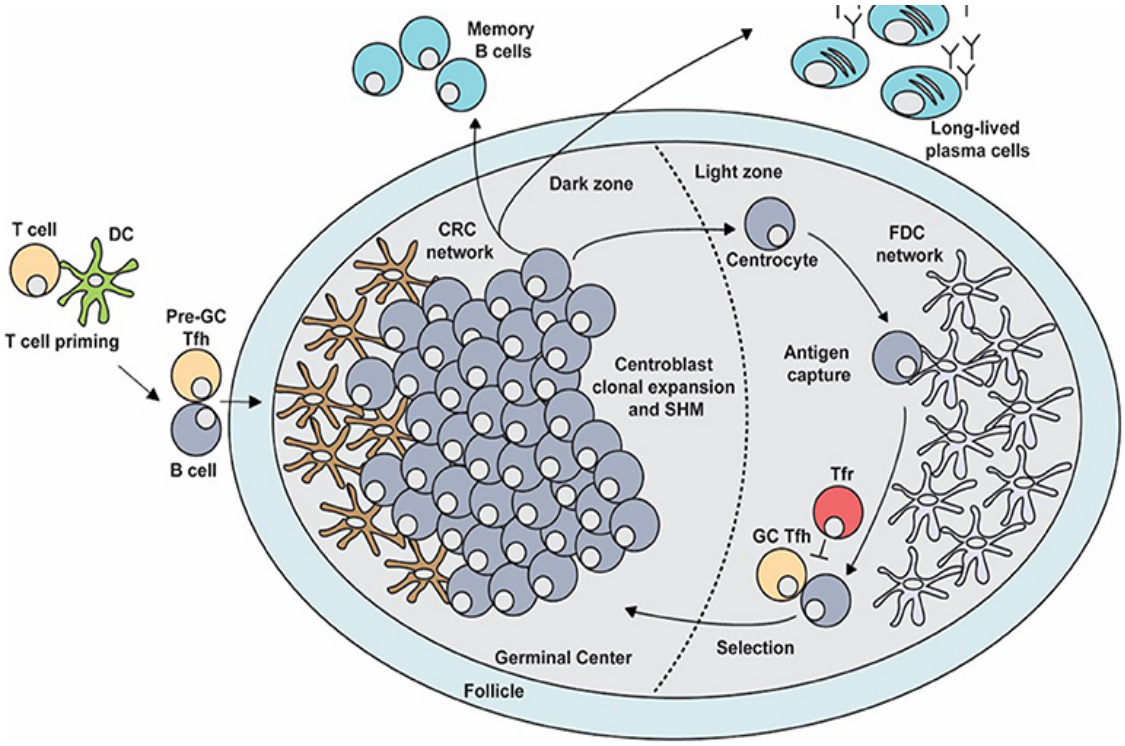

Figure 4 Somatic hypermutation selection of B cell in germinal center. High-affinity B cells clones get help from Tfh cell and return back to dark zone for proliferation. In contrast, low affinity will die by apoptosis. Taken from #131.

1.3.2.3 B cell effector functions

In the adaptive immune response, B lymphocytes recognize antigens to initiate a response and perform their roles. The most important function of B cells is antibody production. An antibody molecule comprises two identical heavy chains and light chains. Disulfide bridges connect these four chains to form a Y-shaped molecule[113]. A light chain contains a variable and a constant domain, and a heavy chain has a variable and three constant compartments. An antibody is composed of two antigen-binding sites in the variable regions. Functionally, antibody molecules can be divided into two identical Fab regions capable of binding antigens and one Fc region mediating effect function and biological activity. There are five distinct types heavy chain, μ, δ, γ, ε and α, which were identified based on the constant regions[113, 137]. Antibodies isotypes, including IgM, IgD, IgG, IgE, and IgA, are designated according to the heavy chains with different biological functions by binding to specific Fc-receptors.

PCs produce antibodies and induce antibody-mediated effector functions. Antibodies enable to neutralize pathogens, enhance phagocytosis and activate the complement system. Moreover, antibodies can recruit and bind with cytotoxic CD8 T cells in an ADCC manner, like NK cells. Finally, anti-pathogen antibodies can trigger mediator release from the granulocytes through

degranulation. As B cells get activated and start the differentiation path to become PCs, they can also produce cytokine, such as IL-10.

1.4.2.3 Autophagy in B cells

When B cells get activated, they go through a program of differentiation that involves changes in metabolism and activation of autophagy. Autophagy is a metabolic process in cells that affects intracellular organelles and long-lived protein to produce energy and metabolic intermediates. Thus, it maintains cell survival during nutrients depletion. It can be divided into three classes: macroautophagy, microautophagy, and chaperone-mediated autophagy[138]. Macroautophagy is the predominant type and most studied. An extended cytosolic membrane encloses ubiquitinated cytosolic fragments to generate double-membrane vesicles called autophagosomes. These matured autophagosomes eventually fuse with lysosomes for autolysosomes which degrade the engulfed cellular compartment. In microautophagy, the degradation happens in connection to the lysosomal membrane. During chaperone-mediated autophagy, cytoplasmic chaperones identify KFERQ motif promote induction of long-lived proteins[139-141]. The autophagy path can be divided into three different stages: initiation, elongation, and completion. Initiation of autophagosome requires the complex interplay between class III PI3 K and autophagy-related genes (Atg)[142, 143]. The extension engaged in ubiquitin-like conjugation, including Atg8/MAP-LC3/GABARAP/GATE-16 and Atg12. The phosphatidyl ethanolamine is then covalently be tied to a lipidated protein called LC3-II. Upon completion, LC3 keeps in the autophagosomal lumen, while Atg12–Atg5–Atg16 complex will be cleaved off. The matured autophagosome combines with lysosome for protein degradation[144].

Autophagy serves a critical role in B cell development. Mice lacking *ATG5* in B cells fail to switch from pro- to pre-B cells in the bone marrow and maintain B-1a B cells in the periphery[145]. Studies further demonstrate that autophagy is essential in B cell activation, PC formation, and humoral autoimmune responses[146, 147]. After BCR or CD40 *in vitro* activation, FOB cells switch from canonical to non-canonical, which mimics the GC response *in vivo*. The following transition of GC B cells into PCs involves returning to canonical autophagy. WD repeat domain phosphoinositide interacting 2 (WIPI2) is highly expressed in GC B cells and interested in this process. WIPI2 deficiency leads to impairment in the transition of canonical to non-canonical manner, decreased GC B cells and PCs[148]. Some reports also

show that pattern recognition receptors drove autophagy to promote an immune response in preventing against microbes[149, 150].

1.4.3 Invariant natural killer T cells

*i*NKT cells are defined using Vα14Jα18 chains for TCRs in mice and part of the unconventional T cell pool. Just like other T cells, *i*NKT cells inhabit lymph nodes and spleens. They migrate to the follicular borders in lymph nodes and spleens to interact with antigen presentation cells during activation. As innate-like lymphocytes, *i*NKT cells recognize glycosphingolipid antigen presented by molecule CD1d. CD1d molecule looks like MHC class I and is expressed by antigen presentation cells. There are five distinct CD1 molecules in humans, including CD1a, b, c, d, and e, while in mice, CD1d is the only lipid presentation molecule[151-153]. Instead of Vα14Jα18 chains for TCRs, type II NKT cells display more varying antigen receptors than *i*NKT cells and recognize hydrophobic antigens like sulfatide[154]. The glycosphingolipid α-galactosylceramide (α-GalCer) is one of the most potent activation agonists for *i*NKT cells. *i*NKT cells and play a significant role in regulating and maintaining humoral immune responses when glycolipid antigens are presented. An example of this is that in *i*NKT cell-deficient $J\alpha18^{-/-}$ mice, there is a failure to produce antibodies when the mice are immunized with antigen coupled to the glycolipid α-GalCer[155].

Signaling between *i*NKT cells and antigen presentation cells is mediated by molecules: CD1d-TCR, CD40-CD40L, OX40-OC40L, and cytokines secretion: IL-2, IL-4, IL-17, IFN-γ, TNF-α, etc. CD1d is expressed by B lymphocytes, macrophages, monocytes, and DCs[156]. CD1d internalizes lipid antigens and traffics them to endosome and lysosome. After activation, *i*NKT cells differentiate into effector cells and assemble as the function of Th1, Th2, Th17 cells, Tfh cells, and cytotoxicity T cells[157]. As innate-like lymphocytes, *i*NKT cells connect the innate and adaptive immune system and play a key role in anti-microbial, anti-tumor, and autoimmune responses. *i*NKT cells provide two different types of help to B cells, including cognate or noncognate help[158]. It relies on features of antigens co-immunized with α-GalCer.

Immunization of α-GalCer targeted to B cells induces cognate *i*NKT cell help. Chemical haptens conjugated with αGalCer like NP-αGalCer are taken up and presented by B cells to produce antigen-specific antibodies[155, 159]. Also, α-GalCer alone can promote iNKTfh cell differentiation as a cognate help to B cells, an immature GC response, and antibody response[160, 161]. The interaction between *i*NKTfh cells and B cells is essential for cognate

help and mediated via CD1d (**Figure 5**)[155, 158]. For example, transgenic MD4 B cells cannot elicit HEL-specific B cell proliferation in Jα18$^{-/-}$ mice after α-GalCer immunization[159].

After coadministration of protein antigens with α-GalCer, *i*NKT cells provide noncognate help

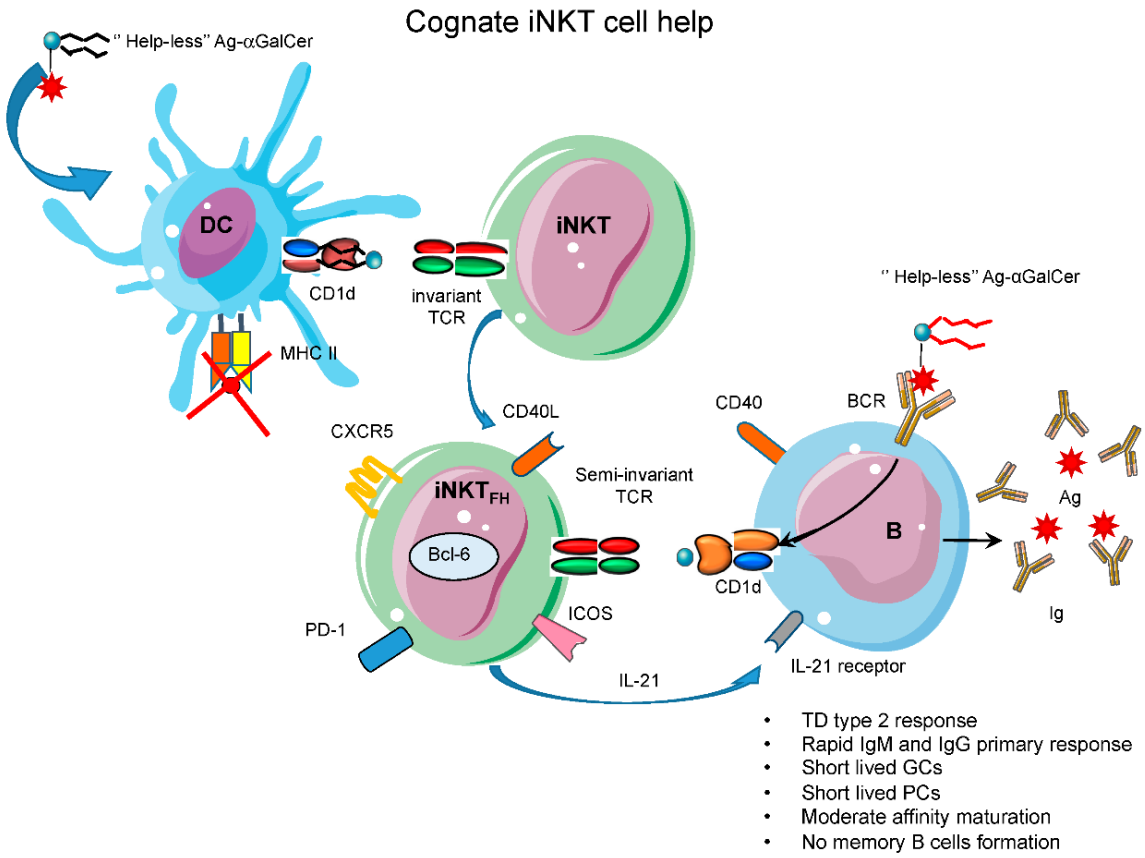

Figure 5. Cognate *i*NKT cell help for B-cell immune responses. For unassisted antigens, *i*NKT cells are in the provision of cognate help to B cells. αGalCer presented by CD8α^{+} DCs will induce *i*NKT cells activation and become *i*NKT $_{FH}$ cells. Through Ag-αGalCer complexes, the *i*NKT $_{FH}$ cells can provide help to B cell and result in TD type 2 B-cell response. Taken from #158.

to B cells and induce a long-lasting antibody response. After the immunization of α-GalCer together with protein antigens, *i*NKT cells are activated by DCs. The activated DCs will relocate to the edge of T and B zones. Protein antigens will be presented to MHC II, and internalized α-GalCer will be displayed to CD1d. DCs provide activation signaling to both *i*NKT cells and T cells. The *i*NKT cells will differentiate into *i*NKTfh cells, which give a cognate helper to B cells[162, 163]. These B cells will proliferate and induce ligand expression for APRIL and activation factor BAFF, essential for the B cell response for long-term maintenance (**Figure 6**)[158, 164]. Consequently, the noncognate immune response will promote the formation of GC, long-lived PCs, memory cells, antibody class switching, and affinity maturation.

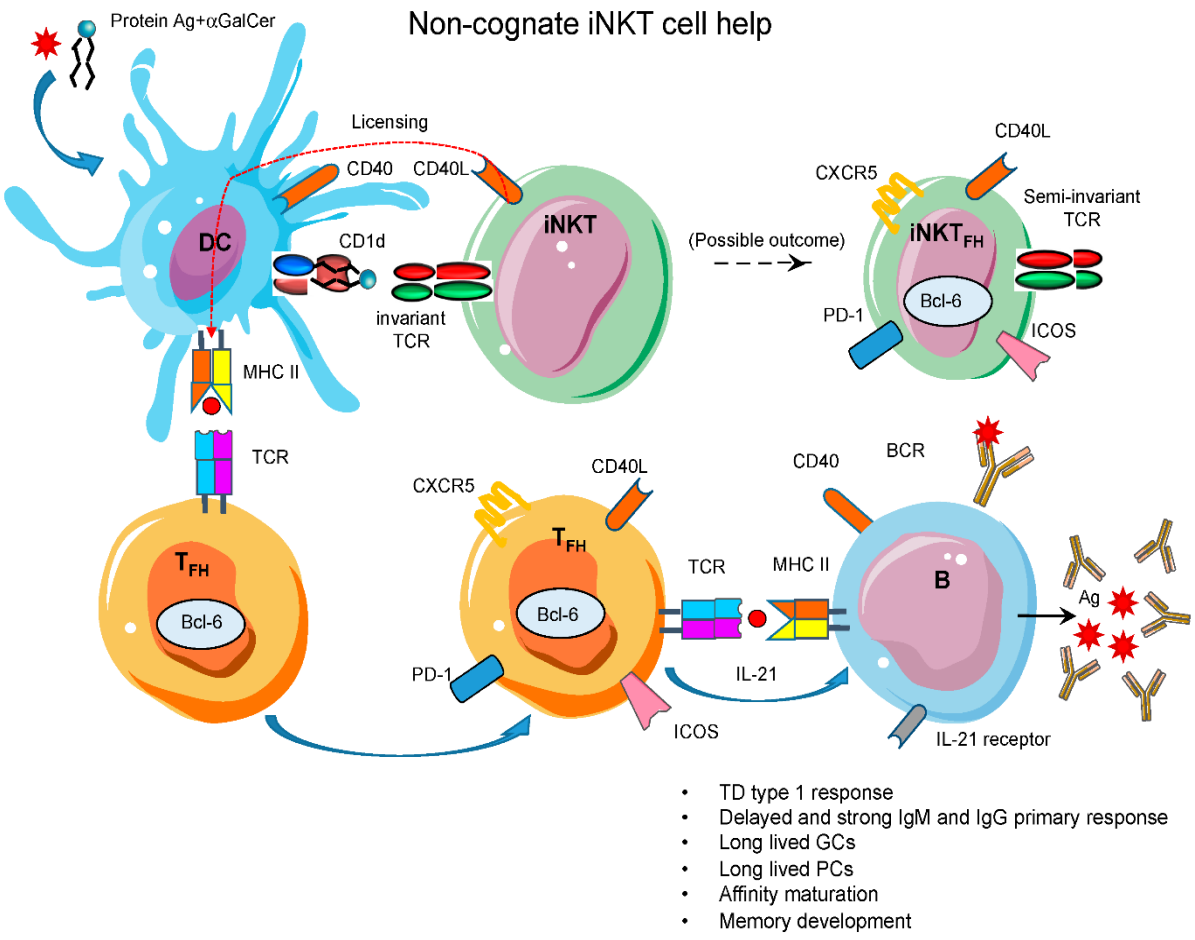

Figure 6. Non-cognate *i*NKT cell help to B cells. Under the co-immunization of protein antigens with αGalCer, *i*NKT cells will be induced non-cognate help to B cells. Recognition of protein antigens by convential $CD4^+$ Tfh cells lead to a typical TD immune response. The interaction of αGalCer-CD1d initiate *i*NKT cell activation. Taken from #158.

Moreover, costimulatory molecules CD80/CD86, CD40, and the production of IFNγ play an essential role in regulating the production of antibodies in cognate activation[155]. However, it has been shown that $CD4^+$ T cells are dispensable for *i*NKTfh cells to provide cognate help to B cells[159]. Thus, in cognate help, the effector helper *i*NKT cells are dominated by *i*NKTfh cells alone[165]. *i*NKTfh cell is similar to the conventional Tfh cells largely for their phenotype and function[160]. Upregulation of transcription factor Bcl-6 and follicle-homing chemokine receptor CXCR5 is the crucial regulator for forming *i*NKTfh cells. Like Tfh cells, *i*NKTfh cells express PD-1, CD40L, and ICOS essential in B cell and *i*NKT cell interaction during an immune response[160, 166]. The cognate help to B cells from *i*NKTfh cells is also inhibited by SAP or CD28 depletion[160, 167]. *i*NKTfh cell differentiation also requires interaction with B cells similar to Tfh cells[160]. The expression of CD40 and CD1d is required on B cells to get cognate help from *i*NKTfh cells[160, 165]. *i*NKTfh cells contribute to form antigen-specific GC B cells and PCs[155, 160]. Furthermore, IL-4, IL-21, and IFN-γ secreted by *i*NKTfh cells are required *i*NKT cells and B cell interaction for their secretion[160, 168].

In the *i*NKTfh cell-mediated GC response, GC B cells will be generated, but they are not sustained and immature compared to the response supported by Tfh cells. Consequently, the response gives limited isotype switching, and antibodies dependent on *i*NKTfh cells also have a lower binding affinity. The PCs have a shorter half-life than Tfh mediated GC responses[160].

It has been shown that α-GalCer activated *i*NKT cells are unresponsive to restimulation. Normally, B cells and T cells can recall memory to re-challenged antigens during adaptive immune responses. Unexpectedly, the *i*NKT cells fail to respond to re-challenged α-GalCer, probably due to a lack of proper co-stimulation. After α-GalCer administration, there is the rapid loss of tetramer-positive *i*NKT cells due to downregulation of TCR that lasts about 24h[169, 170]. Three days after injection, however, *i*NKT cells expand to the maximum. Subsequently, most *i*NKT cells undergo apoptosis and go back to steady-state numbers seven to ten-day after immunization[169, 171]. These *i*NKT cells become anergic for several months[172]. The unresponsive *i*NKT cells also do not produce IL-2 after re-stimulation, which is essential for *i*NKT cell expansion[172, 173]. The exogenous IL-2 stimulation breakthrough *i*NKT cells anergy bypass the TCR signaling. Anergic *i*NKT cells are impaired in killing cells in a melanoma model but can exhibit their effector functions in preventing autoimmune encephalomyelitis (EAE)[172].

1.5 Autoimmune disease

Although the immune system protects us against foreign antigens, it occasionally attacks our tissues resulting in autoimmune disease. Autoimmunity is predominantly mediated by autoreactive B cells and T cells and includes memory. Examples of autoimmune diseases include systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS), inflammatory bowel disease (IBD), and type I diabetes.

The immune system works coordinately to avoid an autoimmune response, and several mechanisms have evolved to achieve this. In the early development, central tolerance mechanisms in primary lymphoid organs for B cells and T cells are important. Central tolerance is achieved by eliminating self-reactive lymphocytes in the primary lymphoid organs during development. In central tolerance, elimination is often the first step in cell development. Through genetic rearrangement of DNA at the variable region and random nucleotides at the junctions, genetic diversity is induced in both B cell and T cell receptors[174]. Some variable regions will inevitably react with self-antigens. If all of them could develop into mature lymphocytes, autoimmune diseases would occur. Luckily, most autoreactive lymphocytes will

be eliminated in the bone marrow and thymus before their maturation[175, 176]. Lymphocytes with high-affinity TCRs and BCRs binding self-antigens will become anergic or apoptosis in primary lymphoid organs, and this process is called negative selection[177-179].

Although most immature lymphocytes with strong self-reactivity will be deleted in central lymphoid organs, some clones with weak self-reactivity can survive. Outside primary lymphoid organs, peripheral tolerance occurs and inhibits autoreactive lymphocytes. Peripheral tolerance is active and specific immunosuppression. For both B cells and T cells, it relies on immune cells with immune-inhibitory or regulatory function[180]. Treg cells are the most well-known in immune suppression, while subsets of B cells, DCs, macrophages, and myeloid-derived suppressor cells (MDSC) are also involved in regulation[179-182].

Both CD4 and CD8 lymphocytes are regulated to avoid autoimmunity. Upon activation, TCRs on CD4 T cells will bind with antigens presented by MHC (signal 1). At the same time, T cells engage in the interaction between CD28 on T cells and CD80/CD86 on the antigen presentation cells (signal 2)[183]. CTLA-4 is also bound with CD80/CD86, inhibiting T cell activation, thus providing negative co-stimulation. The importance for this was shown in mice deficient for CTLA-4, which have enormous lymphocyte expansion and spontaneous autoimmune disease[184]. Activation of $CD4^+$ Treg cells includes upregulation of the FoxP3 transcription factor, CTLA-4, and IL-2R α chain[185, 186]. People who inherited X-linked gene mutations in FoxP3 result in a multiorgan autoimmune illustrating the importance of this regulation [187]. It has been shown that $CD4^+$ Treg cells play a critical role in graft rejection and also transplantation settings [188, 189]. Compared with $CD4^+$ Treg cells, $CD8^+$ Treg cells are less well characterized, less frequent, and described to have a more diverse phenotype[190]. Except for the expression of CTLA-4 and FoxP3, $CD8^+$ Treg also highly express αα chains rather than α and β chains[191, 192]. The best-characterized $CD8^+$ Treg cells are activated by nonclassical MHC I in humans and HLA-E Qa-1 mice[193, 194]. Adoptive transfer of $CD8^+$ Treg cells protects mice from experimental autoimmune encephalomyelitis (EAE)[195, 196]. Treg cells reduce the costimulatory signals through surface molecules and cytokine secretion. An important mechanism is the downregulation of CD80/CD86 on the antigen presentation cells, possibly leading to immunosuppression by leveling the degree of activation[181].

Without T cell help (second signal), B cells become anergic and do not enter the GC if they re-encounter their antigen. Therefore, the T-cell self-tolerance regulates peripheral tolerance of B-cell as well[197]. Many experiments have also highlighted the importance of Bregs and the inhibitory cytokine IL-10[198]. Mice lacking IL-10 secretion from B cells have a higher Th1

cell activation and a worsening of multiple sclerosis and rheumatoid arthritis[199, 200]. In humans, Bregs have been shown to be involved in disease activity of rheumatoid arthritis and lupus erythematosus[198, 201-203]. Another regulatory cell is the MDSC. They secrete inhibitory molecules like IL-10, arginase-1, inducible nitric oxide synthase (iNOS) and express the negative costimulatory molecule PD-L1 negatively modulate T-cells expansion[182].

During the fetal and early neonatal stage, exposure to antigen results in suppression response as the immune system is set to become tolerant to the new environment[204]. Except for fetal exposure, factors that promote tolerance include exposure to large amounts of antigens, long-time treatments with antigens, intravenous administration, absence of adjuvants, display of antigen by activated macrophages, DCs, and B cells. This is used in immune therapy of allergic individuals for treatment where the goal is to increase tolerance and reprogram the immune system.

SLE is one of the most well-known examples of systemic autoimmune disease. Like many other autoimmune diseases, SLE is more common in females than males[205]. SLE symptoms typically appear at age 20-40 and are more prevalent in Hispanic and African Americans than other populations[206]. Genetic factors contribute to the occurrence of SLE as studies show that identical twins have a 60% risk of developing SLE if the other twin already was affected[207]. SLE patients suffer from type III hypersensitivity due to the formation of autoreactive immune complexes. These complexes initiate the complement cascade and form membrane-attack complexes that cause damage to blood vessels[208, 209]. In severe cases, large immune complexes can drive neutrophil accumulation and attachment to vascular endothelium and cause the spread of tissue damage[210]. SLE patients are diagnosed with anti-nuclear antibodies or indirect immunofluorescence to stain autoreactive human cells.

Several animal models have been used in SLE studies to provide insights into the genetic contribution to the disease. The most well-known spontaneous model is the F1 mice of New Zealand Black (NZB) crossed with New Zealand white (NZW). These mice produce autoantibodies and develop glomerulonephritis[211]. MRL/lpr mice are the other commonly used mice strain produced by hybridizing mice strains, including B6, C3H, AKR, and LG[212, 213]. In MRL/lpr mouse, the Fas receptor gene mutation causes abnormal apoptosis in B cells and T cells[214, 215]. A population of double-negative T cells is generated in the mouse, which results in lymphadenopathy and splenomegaly[216, 217]. MRL/lpr mice produce autoantibodies and develop skin rash, vasculitis, and arthritis[218-220].

Apart from spontaneous autoimmune models, induction models are commonly used in SLE studies. One of the well-characterized is the pristane-induced model. As a mineral oil, pristane injection induces SLE-related antibodies and lupus-like disease[221]. Resiquimod cream administered to the specific mice ears can also trigger SLE-associated disease after administration for 2-4 weeks. Due to the TLR7 ligand in the cream, the mice upregulate type I IFN and drive the condition [222, 223]. It is known that apoptotic cell clearance has a vital role in the SLE development in patients. Our studies employ a model with apoptotic cell injections to initiate SLE-like disease and break tolerance[224, 225]. The administered mice produce antibodies against ssDNA, cardiolipin, nuclear proteins and develop mild glomerulonephritis. A memory formed in the model where a boost administration can recall the immune response to apoptotic cells, and memory response is transferable to naïve mice[225]. Using this model in mice deficient in different tolerance checkpoints gives an enhanced disease. This model highlights that proper clearance of apoptotic cells is needed to avoid autoimmune disease.

1.5.1 B cells in autoimmunity

B cells play an essential role in modulating the pathogenic mechanism of autoimmune disease. Autoantibodies often are a driving force of autoimmune disease in patients[103, 226] [227]. A low level of autoantibodies can be detected in healthy individuals, suggesting that central and peripheral tolerance is not complete[228, 229]. A mild self-reactivity is required to maintain healthy and helps to clear cellular debris, whereas the self-reactive antibodies induce tissue damage during autoimmune disease. Thus, antibodies are generally sticky, a feature that is often described as being polyreactive.

If B cell tolerance is broken and PCs are formed, they establish the autoimmune disease by producing switched autoreactive antibodies and generating memory. Shorted-lived PCs will be developed in the extrafollicular area, whereas long-lived PCs are created through GC responses[230]. PCs display various homing receptors based on the inflamed sites and migrate back to the infected sites[231, 232]. Here, inflammatory cytokines extend the lifetime of homed PCs and sustain antibody production[233, 234] [235]. PCs can be found with different phenotypes and various differentiation stages in autoimmune diseases. Some PCs can express CD20 and are then susceptible to rituximab treatment, where antibodies are used to deplete B cells[236, 237]. In contrast, some mature PCs migrated to the bone marrow do not express CD20 and are depleted[238].

Autoreactive memory B cells will be generated in GC response during an infection. Some autoreactive memory B cells exhibit IgM expression, while a significant percentage switched to IgG or IgA. Human memory B cells upregulate the expression of CD27, which binds to CD70 on T cells to help with their reactivation. The interaction enhances the transfer from memory B cells into functional PCs and strongly promotes antibody production even with a small degree of stimulation[239]. Autoreactive memory B cell formation is a crucial element in many autoimmune diseases and contributes to disease activity flares. However, memory B cells are sensitive to Rituximab treatment, depleting all memory B cells[240],[241].

1.5.2 B cell-directed immunotherapy

Apart from Rituximab (anti-CD20), monoclonal antibodies against CD19, BAFF, CD22, and CD40 are used to treat autoimmune diseases.

The expression of CD20 begins at the pro-B cell stage and increases in the maturation process. CD20 modulates B-cell activation, proliferation, and differentiation through the regulation of transmembrane Ca^{2+} [242]. Therefore, monoclonal antibodies against CD20 like Rituximab are efficient clinical therapy methods for autoimmune disease by inhibiting B cell activation[243].

Of the other targets, CD19 is expressed on B cells, including pre-, immature, and mature B cells[244]. Therefore, CD19-direct therapies can also deplete early precursor, pre-, immature B cells mature B cells, peritoneal cavity B cells, pathogenic B cells, and inhibit humoral immune response[245, 246]. Like anti-CD20, CD19 mAb eliminates B cells via antibody-mediated cytotoxicity[245].

Another way to target B cells is through the cytokine BAFF, secreted by DCs, macrophages, neutrophils, and Tfh cells. This treatment is specific as BAFF receptors are mainly expressed by B cells. As a TNF family member, BAFF has a vital function in initiating the activation and preventing B cells from undergoing apoptosis. This is especially important during BCR ligation, where BAFF provides survival signals[247-249]. Thus, targeting BAFF has shown to be vital immunotherapy to suppress autoreactive B cells. Clinical data show that belimumab, a BAFF antibody, results in B-cell depletion in SLE patients[250],[251]. It has been approved in the treatment of SLE in many countries.

Finally, the costimulatory molecule CD40 is upregulated in B-cell development and provides a survival signal for B cells. CD40 ligand is predominantly expressed by CD4 T cells. *In vitro*, the combination of anti-CD40 antibodies and IL-4 upregulates the early activation marker

CD69 and CD154, elevates expression of CD80, CD86, MHC-II, CD95, and adhesion molecules VLA-4, and enhances the secretion of IL-6, TNF-α, and chemokines on B cell[252]. The interaction between CD40 and CD40 ligand enhances B cell activation, proliferation, and differentiation in the adaptive immune response. Humanized CD40 ligand and CD40 mAb used for treatment in targeting this pathway result in decreased serum level against double-strand DNA antibodies in SLE patients[253-255].

1.5.2 Invariant nature killer T cells in autoimmunity

*i*NKT cells induce immune responses against pathogens, but their role remains controversial in autoimmunity. Reduced iNKT cells in patients with autoimmune disease suggest that *i*NKT cells can have a protective role[256]. This is also consistent with other observations. Aging mice exhibit a deficiency of *i*NKT cells connected to autoimmune symptoms[257]. Mice deficient iNKT cells do not develop spontaneous autoimmune disease[256, 258].

The different roles of *i*NKT cells in autoimmunity may be associated with different *i*NKT cell subsets and activation modes. When the inflammatory cytokine IL-18 is injected alone, iNKT cells block autoreactive B cell activation. This is mediated by interactions with neutrophils that license the NKT cells[259]. In contrast, if IL-18 is co-injected with glycolipid triggering *i*NKTfh cells, which enhance autoreactive GC response[161]. This could be the reason for α-GalCer accelerated autoimmune in lupus mice models. In contrast to this, other studies show that the interaction of *i*NKT cells and Bregs inhibits the autoantibody responses[260-263]. Moreover, *i*NKT cells and CD1d inhibit autoreactive B cell activation in mice models using apoptotic cells induce autoimmune, which may also influence the formation of Bregs[264]. Collectively, *i*NKT cells are a double-edged sword in regulating autoreactive B cells, and the type of regulation is context-dependent.

2. AIMS

Paper I To investigate a possible role of scavenger receptor CD36 initiated autophagy in B cell regulated T-dependent humoral immune responses.

Paper II To investigate the role of endosomal pattern recognition receptors in apoptotic cell mediated autoimmune response.

Paper III To reveal the balance between conventional and unconventional follicular helper T cells direct autoreactive B cells.

3. RESULTS AND DISCUSSION

3.1 CD36 and LC3B initiated autophagy in B cells modulates the humoral immune response (Paper I)

Scavenger receptor CD36 plays an essential role in innate immune responses[26]. The role of CD36 has been described in macrophages but remains less explored in B cells. This study finds that CD36 mediated autophagy in B modulates T-dependent immune response.

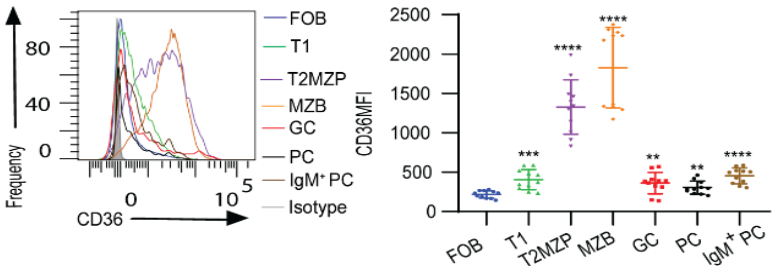

Figure 7. CD36 staining in B cells from spleen of C57BL/6 mice. Taken from paper I.

To study the role of CD36, we characterized the expression pattern of CD36 in different B cell subtypes. The highest expression was found in MZB and T2 MZP cells, followed by T1, GC, PC, IgM$^+$ PC B cells (**Figure 7**). *In vitro*, the expression of CD36 in B cells was induced after stimulation with LPS, CPG, or anti-CD40+IL-4. The elevated CD36 level was found on the cell surface and intracellularly. We also show that B cells lacking CD36 formed fewer PCs than WT cells in *ex vivo*.

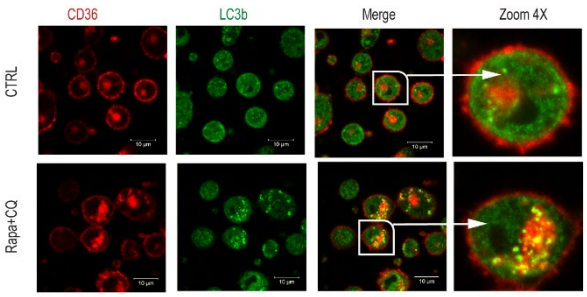

Figure 8. CD36 colocalized with LC3B under autophagy induction. Taken from paper I.

Next, we screened mouse B cell lines to study the role of CD36 mechanistically and found that CD36 was highly expressed in CH12 cells, a lymphoma cell line[265]. Using immunoprecipitation and mass spectrometry, we found that CD36 interaction proteins were involved in metabolism, and many of these were located in the endoplasmic reticulum and mitochondria. Depletion of CD36 in CH12 cells led to less formation of autophagosomes. After autophagy induction using rapamycin and chloroquine, CD36 was colocalized together with LC3B on autophagosomes. Co-immunoprecipitation was used to evaluate the colocalization of CD36 and LC3B. It showed that LC3B-II was co-immunoprecipitated with CD36 after the stimulation with rapamycin and chloroquine (**Figure 8**).

Given that CD36 was involved in forming PCs and in B cell autophagy, we performed additional *in vivo* experiments. It has been reported that CD36 deficiency mice produce fewer antibodies against *Streptococcus pneumonia*[26], but the role of CD36 in T-dependent antigen

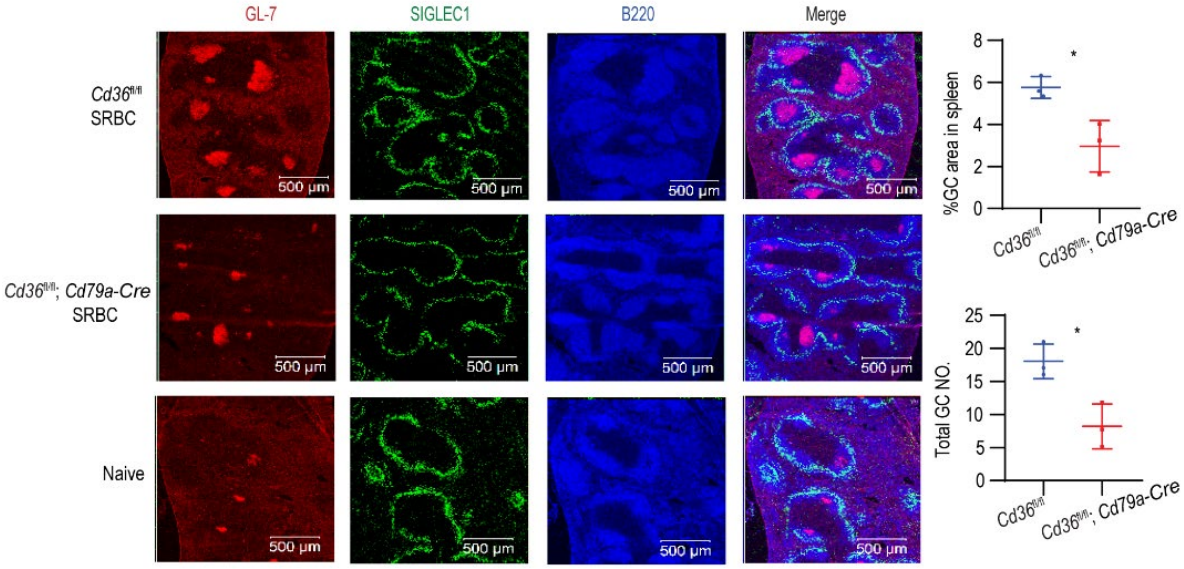

Figure 9. Mice with CD36 depletion in B cell selectively impaired T-cell-dependent immune response. Taken from paper I.

responses and contribution in different cell types remains unknown. To assess the role of CD36 specifically in B cells, we crossed mice carrying floxed *Cd36* alleles (*Cd36*$^{fl/fl}$) with mice expressing *Cd79a/Mb1Cre* recombinase generating mice with specific deletion of CD36 in B cells[266, 267]. Sheep red blood cells (SRBCs) were used to immunize these mice as a model T-dependent antigen. The formation of GC B cells, Tfh cells, PCs, and antibodies were used to evaluate the immune response (**Figure 9**). In the GC response, we observed less formed GC B cells and Tfh cells in mice lacking CD36 in B cells. Mice with CD36$^{-/-}$ B cells also developed fewer overall PCs and class-switched PCs. The reduction of the formation of GC B cells and PCs also led to fewer antibodies against SRBCs in mice lacking CD36 B cells. We also confirmed the *in vitro* data and found that knockout mice had reduced the formation of autophagosomes in GC B cells. Using *Cd36*$^{fl/fl}$ *Aicda-Cre* mice[268], we showed that CD36 expression in GC B cells partly contributed to the immune response to SRBCs. However, no difference was detectable between WT mice and mice lacking CD36 B cells after immunization with the T-independent type I antigen NP-LPS or the type II antigen NP-Ficoll. This shows that CD36 in B cells was required for T-dependent immune response and autophagosome formation. Collectively, this study reveals a novel role of CD36 in autophagy to support the GC response in T-dependent immune responses.

3.2 The B cell response to both protein and nucleic acid antigens displayed on apoptotic cells are dependent on endosomal pattern recognition receptors (Paper II)

As pattern recognition receptors, TLRs serve a critical role in an innate immune response connected to infection. TLR3/7/9 receptors can sense DNA and ssRNA and are highly expressed by antigen presentation cells, including B cells, macrophages, and DCs. Crossing TLR7 or TLR9 mice with MRL/Mp$^{lpr/lpr}$ mice has demonstrated that TLR 7 and 9 were involved in the autoantibodies production[269]. However, it is unknown if these TLRs contribute to response against all autoantigens beyond DNA and RNA and related proteins. We used mice with a mutation in the Unc93b1 (3d) gene that lacks the function of TLR3/7/9. We know from previous studies that consecutive immunization with apoptotic cells initiates SLE-like

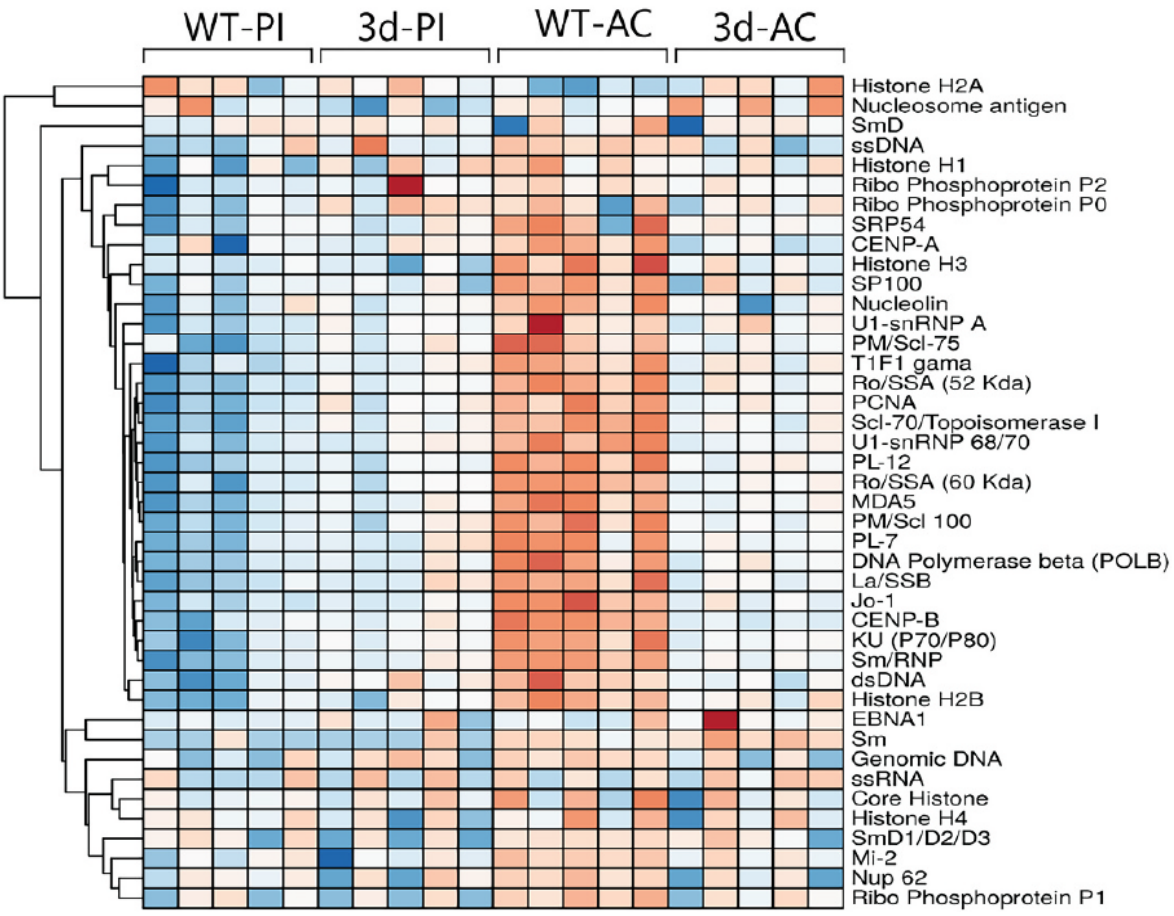

Figure 10. Heat maps showing autoantibody array. Taken from paper II.

phenotype without severe pathology. Thus, we immunized 3d mice and WT mice with ACs weekly four times to induce an autoimmune response. We found that autoantibodies were produced against both nucleic acids, including DNA, histone, SmD1, Ro-52, Ro-60, La, and unrelated antigens like Cardiolipin Phosphoryl Choline. To identify an even broader spectrum of autoantibodies, we used an autoantigen array with 128 autoantigens. No difference between

WT and 3d mice was observed without immunization in background levels of antibodies in serum. However, after injection, the data shows that autoreactive IgG against nuclear, circulating, membrane and cytoplasmic antigens were diminished in 3d mice compared to WT mice. This was true for both IgM and IgG antibodies showing that depletion of endosomal TLRs reduced all types of reactivities (**Figure 10**). The formation of PCs is mediated through extrafollicular and follicular GC responses. Short-lived IgM-producing PCs are predominantly found by extrafollicular responses, while the long-lived and class-switched are preferentially from GC responses[270]. We measured IgM and IgG antibody responses at different time points to investigate the kinetics and role of endosomal TLRs. Apoptotic cell injections led to elevated autoantibodies against DNA, cardiolipin, and other autoantigens. The 3d mice had reduced production of autoantibodies and GC formation. The reduction in GC formation was accompanied by decreased Tfh cells in the 3d mice. Next, PC responses and effects on class-switching were assessed. After injections, we found decreased IgM^+, $IgG1^+$, $IgG2^+$, $IgG3^+$ PCs in the 3d mice compared with WT mice. We also investigated the generation of age-associated B cells (ABCs) that arise in old humans and mice and chronic inflammation. They have been shown to contribute to autoimmune disease and are defined by the expression of transcription factor T-bet and surface marker CD11c[271]. We found that in the 3d mice, there was also a reduction in the formation of ABCs compared to WT mice. Thus, we conclude that both auto-antibody responses and generation of B cell phenotypes connected to chronic inflammation were dependent on endosomal TLR signaling.

Endosomal TLRs are expressed by both DCs and B cells and could contribute to the activation of these and other cell types in this model[272]. To understand the intrinsic role in B cells, we made mixed BM chimeras generated by a 1:1 mixture of WT (CD45.1): WT (CD45.2) or WT (CD45.1): 3 d (CD45.2) bone marrow. Two months after transplantation, the mice were injected with apoptotic cells as in previous experiments, and immune responses were evaluated. The differentiation into GC B cells and PCs from 3d (CD45.2) was 2-fold lower than that of the CD45.1 allotype. This shows that GC B cells and PCs from 3 d mice cells expanded less and were not as activated compared to WT cells. No difference between WT and 3 d bone was detected for the reconstitution of B1 cells, MZBs, FOBs, and T cells. The data shows that TLRs deficiency, specifically in B cells, drives the phenotype. We assessed signaling differences between WT and 3d mice B cells after $F(ab)_2$ anti-IgM induction. We found that BCR-induced calcium signaling was comparable between wild-type and 3d B cells. Moreover, to show the specificity of endosomal TLRs to stimulation, splenic and peritoneal B cells were stimulated

with LPS. No difference was found between WT and 3 d mice in cell proliferation and apoptosis. Collectively, endosomal TLRs deficiency mice respond comparably as WT mice to BCR and TLR4 stimulation, suggesting that the cells developed normally. The phenotype was directly connected to the response induced by apoptotic cells. In summary, the data shows that TLR3/7/9 are essential in self-tolerance and serve as a critical link between innate immunity to autoimmunity.

3.3 The balance between convention and unconventional follicular helper T cells direct autoreactive B cells (Paper III)

*i*NKT cells induce a quick immune response to foreign and self-antigens and can have a dual function in regulating B cell responses. How *i*NKTfh cells contribute to autoimmune response is poorly characterized. Here, we investigate this in the apoptotic cell model induced break of B cell tolerance and use the *i*NKT cells agonist α-Galcer to shift the function of iNKT cells from regulators to helpers. Mice were injected with vehicle or ACs alone four times or AC+α-GalCer for the last two injections. Co-injection activated both *i*NKTfh and Tfh cells, but both

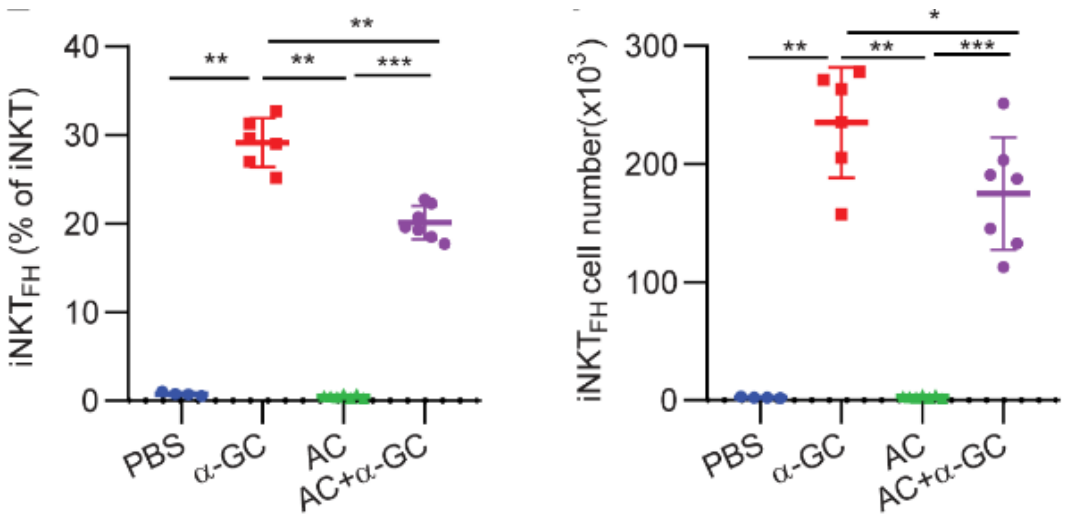

Figure 11 Immunization with α-GalCer triggers the generation of *i*NKTfh cells. Representative contour plot showing the frequency and accurate number of splenic *i*NKTfh cells.

populations were minor than α-GalCer alone or AC alone (**Figure 11**). As proof of activation increased, Bcl-6 and CD40L were expressed by *i*NKTfh cells relative to CXCR5$^-$PD-1$^-$ *i*NKT cells. To investigate how this balance between *i*NKTfh and Tfh cells affected the response, we examined GC B cells and PCs formation. We found that mice immunized with AC+α-GalCer formed more GCs B cells than ACs alone, and they were located in the follicles. Thus, the combined activation of iNKTfh and Tfh gave a more robust response than AC alone. Also, coadministration elicited less PC and IgG1$^+$ PC than ACs alone, confirmed by immunofluorescence. For long-term response, mice were injected with ACs five times, again

with the addition of a-GalCer for the two last injections, and spleens and serum were collected 14 days after the last injection. Like with the shorter regime, we observed that mice with ACs+ α-GalCer formed fewer GC, $IgG1^+$ GC B cells, and autoreactive IgG1 compared with ACs alone. Next, to investigate whether CD1d on B cells are required in the response, mice carrying floxed *Cd1d* alleles ($Cd1d^{fl/fl}$) were crossed with mice with Cre-recombinase under the control of *Mb1* promotor (*Mb1-Cre*) and produced mice ($Cd1d^{fl/fl}Mb1Cre$) that deletes *Cd1d* specifically in B cells. The glycolipid α-GalCer alone was injected into the mice to investigate cognate *i*NKT cell help, and as expected, we found that $Cd1d^{fl/fl}Mb1Cre$ mice had less B cell activation. The mice lacking CD1d on B cells also resulted in immature GC response and fewer $IgG1^+$ GC B cells. Of importance, we also found fewer *i*NKTfh cells in mice lacking CD1d on B cells, and the ones generated were immature based on less PD-1 expression. Moreover, mice bearing $CD1d^{-/-}$ B cells had less total and Ig class-switched PC than WT mice. Thus, CD1d expression on B cells was needed maturation of *i*NKTfh cells and antibody production. To characterize *i*NKTfh cells mediated help to B cells, we immunized $Cd1d^{fl/fl}$ and $Cd1d^{fl/fl}Mb1$-*Cre* mice with AC+α-GalCer. Mice lacking CD1d on B cells formed fewer GC B cells than WT mice. Tfh cell formation was comparable between WT and KO mice, while the *i*NKTfh cell formation was impaired in mice lacking CD1d on B cells. Further, mice lacking CD1d in B cells led to fewer IgM^+, $IgG1^+$, and $IgG3^+$ plasmablasts. Autoreactive IgG1 against autoantigens from mice bearing $CD1d^{-/-}$ B cells was significantly lower than WT mice. Collectively, mice without CD1d on B cells initiate less *i*NKTfh cells mediated GC response and autoantibody production. Finally, to assess the role of endosomal TLRs in *i*NKTfh cells trigged GC response, we used unc93b mice with deficiency for endosomal TLR3/7/ 9. The data showed that unc93b mice had less GC B and *i*NKTfh cell generation than WT mice, while Tfh cell activation was comparable. In summary, *i*NKTfh cells promote autoreactive B cell formation, and CD1d on B cells and endosomal TLRs perform an essential role in *i*NKTfh cell-regulated autoimmune responses.

4. CONCLUSION AND FUTURE PERSPECTIVES

The balance provided by the immune system has evolved to maintain integrity and health. Abnormal regulation in the immune system can trigger diseases like uncontrolled infection, autoimmune, and cancer. B cells exhibit a critical function in the immune response, including antigen recognition and presentation, immune cell activation, GC response, and production of antibodies. It is essential to know the molecules contributing to B cell function and regulation to restore immune balance. This thesis investigates the role of CD36, endosomal TLRs, and glycolipid presenting molecule CD1d in B cells in the immune response. The significant findings and future perspectives of the thesis are the following:

I. CD36 and LC3B initiated autophagy in B cells regulates the humoral immune response

Paper I reveals that the scavenger receptor CD36 in B cells regulates T-dependent immune response through autophagy. Depletion CD36 in B cells shows defects in forming total and class-switched PCs *in vitro*. CD36 function was associated with intracellular metabolism, including mitochondria, endoplasmic reticulum, cytosolic part, etc. CD36 deficiency B cells led to less autophagosome formation than WT B cells. And we also found that CD36 was colocalized and interacted with LC3 under autophagy induction. Mice without CD36 expression impaired the response to type II T-independent antigens[26]. Using mice conditionally deleted for CD36 in the B cell compartment, we demonstrated that functional CD36 in B cells is required for GC response. These results are significant as they describe a new function for this pattern recognition receptor in B cells. In the future, it will be interesting to investigate if this is also true for other scavenger receptor family members. Also, human B cell lymphoma can express CD36, and it would be interesting to examine if CD36 plays a role in the survival and adaption of these cancer cells.

II. The B cell response to both protein and nucleic acid antigens displayed on apoptotic cells is dependent on endosomal pattern recognition receptors

Paper II reveals that endosomal TLRs regulates autoreactive B cells in autoimmunity. B cell can take up nucleic acids through BCR internalization for endosomal activation of TLRs 3, 7, and 9. The engagement results in a signaling cascade initiation and induces a second signal to activated B cells. Mice that lack endosomal TLRs still generate B1 cells, MZBs, and FOBs. However, they fail to active GC B cell response and form class-switched PCs. The failure of developing GC led to the deficiency in the production of autoantibodies. Thus, paper II

illustrates the importance of TLRs in autoimmunity and their potential role in immunotherapy. In line with paper I, this is excellent proof to show the connection of innate and adaptive immune response by regulating pattern recognition receptors to B cells. Future studies are warranted to uncover the mechanism of endosomal TLRs mediated autoimmunity. Additionally, mice lacking endosomal TLRs, specifically in B cells or DCs, will be needed to clarify the cell-specific roles. An important finding is that all auto-antigen specificities are dependent on endosomal TLRs. Thus, targeting these could be used for targeted treatments and block all autoantibodies classes.

III. The balance between convention and unconventional follicular helper T cells direct autoreactive B cells

Paper III investigates how *i*NKT cells regulate autoimmunity when differentiated into *i*NKTfh cells. Immunization with AC+α-GalCer resulted in quick increasing GC B cells and *i*NKTfh cells and autoantibodies. The results suggest that *i*NKTfh cells modulated the early GC and autoimmune response and impacted the long-term response. After coadministration, we found a balance in T cell help where fewer *i*NKTfh cells were generated than α-GalCer alone and fewer conventional Tfh cells than AC alone. Thus, the data shows the competition between *i*NKTfh cells and conventional Tfh cells upon coadministration. We also found that CD1d on B cells was needed to mature the *i*NKTfh cells fully and form GC response and autoantibodies. The endosomal TLRs in mice were also required for *i*NKTfh cells regulated GC response. Future studies are warranted to uncover the exact mechanism that results in the competition between *i*NKTfh cells and Tfh cells and the *i*NKT cells mediated B cell activation. Several reports investigate the balance between different Tfh populations, and this study shows that activation of unconventional T cell help will direct and modulate the Tfh response. Hopefully, these findings will lead to studies on balance between different types of helper T cells and patients with autoimmunity.

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"Have the courage to follow your heart and intuition."

-Steve Jobs

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6. REFERENCES

1. Sigerist, H.E., *A history of medicine*. Vol. 2. 1987: Oxford University Press.
2. Pasteur, L., *A Centennial Celebration: Pasteur and the Modern Era of Immunization.*
3. Metschnikoff, E.J.B.m.j., *Lecture on phagocytosis and immunity.* 1891. **1**(1570): p. 213.
4. Tiselius, A. and E.A.J.T.J.o.e.m. Kabat, *An electrophoretic study of immune sera and purified antibody preparations.* 1939. **69**(1): p. 119.
5. Lee, M.K., et al., *Interplay between clonal hematopoiesis of indeterminate potential and metabolism.* 2020. **31**(7): p. 525-535.
6. Edition, T.E., *Anatomy and Physiology Volume 2 of 3*. 2014: Lulu. com.
7. Mebius, R.E. and G.J.N.r.i. Kraal, *Structure and function of the spleen.* 2005. **5**(8): p. 606-616.
8. Chatterjee, B., et al., *Internalization and endosomal degradation of receptor-bound antigens regulate the efficiency of cross presentation by human dendritic cells.* 2012. **120**(10): p. 2011-2020.
9. Bermejo-Jambrina, M., et al., *C-type lectin receptors in antiviral immunity and viral escape.* 2018. **9**: p. 590.
10. Janeway Jr, C.A. and R.J.A.r.o.i. Medzhitov, *Innate immune recognition.* 2002. **20**(1): p. 197-216.
11. Lokshin, A., et al., *Adenosine-mediated inhibition of the cytotoxic activity and cytokine production by activated natural killer cells.* 2006. **66**(15): p. 7758-7765.
12. Huang, S., et al., *Role of A2a extracellular adenosine receptor-mediated signaling in adenosine-mediated inhibition of T-cell activation and expansion.* 1997. **90**(4): p. 1600-1610.
13. Suzuki, H., et al., *A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection.* 1997. **386**(6622): p. 292-296.
14. Savill, J. and V.J.N. Fadok, *Corpse clearance defines the meaning of cell death.* 2000. **407**(6805): p. 784-788.
15. Limmon, G.V., et al., *Scavenger receptor class-A is a novel cell surface receptor for double-stranded RNA.* 2008. **22**(1): p. 159-167.
16. Pearson, A.M.J.C.o.i.i., *Scavenger receptors in innate immunity.* 1996. **8**(1): p. 20-28.
17. Lim, K.-H. and L.M.J.C.S.H.p.i.b. Staudt, *Toll-like receptor signaling.* 2013. **5**(1): p. a011247.
18. van Berkel, T.J., et al., *Scavenger receptors: friend or foe in atherosclerosis?* 2005. **16**(5): p. 525-535.
19. Abumrad, N.A., et al., *Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36.* 1993. **268**(24): p. 17665-17668.
20. Asch, A.S., et al., *Analysis of CD36 binding domains: ligand specificity controlled by dephosphorylation of an ectodomain.* 1993. **262**(5138): p. 1436-1440.
21. Guthmann, F., et al., *Ectoprotein kinase-mediated phosphorylation of FAT/CD36 regulates palmitate uptake by human platelets.* 2002. **59**(11): p. 1999-2003.
22. Ho, M., et al., *Ectophosphorylation of CD36 regulates cytoadherence of Plasmodium falciparum to microvascular endothelium under flow conditions.* 2005. **73**(12): p. 8179-8187.
23. Ehehalt, R., et al., *Uptake of long chain fatty acids is regulated by dynamic interaction of FAT/CD36 with cholesterol/sphingolipid enriched microdomains (lipid rafts).* 2008. **9**(1): p. 1-12.
24. Endemann, G., et al., *CD36 is a receptor for oxidized low density lipoprotein.* 1993. **268**(16): p. 11811-11816.
25. Silverstein, R.L. and M.J.S.s. Febbraio, *CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior.* 2009. **2**(72): p. re3-re3.
26. Won, W.-J., M.F. Bachmann, and J.F.J.T.J.o.I. Kearney, *CD36 is differentially expressed on B cell subsets during development and in responses to antigen.* 2008. **180**(1): p. 230-237.
27. Su, X., N.A.J.T.i.E. Abumrad, and Metabolism, *Cellular fatty acid uptake: a pathway under construction.* 2009. **20**(2): p. 72-77.

28. Bonen, A., et al., *Muscle contractile activity increases fatty acid metabolism and transport and FAT/CD36.* 1999. **276**(4): p. E642-E649.
29. Febbraio, M., et al., *A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism.* 1999. **274**(27): p. 19055-19062.
30. Oh, D.S. and H.K.J.A. Lee, *Autophagy protein ATG5 regulates CD36 expression and anti-tumor MHC class II antigen presentation in dendritic cells.* 2019. **15**(12): p. 2091-2106.
31. Sanjurjo, L., et al., *The human CD5L/AIM-CD36 axis: a novel autophagy inducer in macrophages that modulates inflammatory responses.* 2015. **11**(3): p. 487-502.
32. Abumrad, N.A., et al., *Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36.* The Journal of biological chemistry, 1993. **268**(24): p. 17665.
33. Baillie, A.G.S., C.T. Coburn, and N.A. Abumrad, *Reversible Binding of Long-chain Fatty Acids to Purified FAT, the Adipose CD36 Homolog.* The Journal of Membrane Biology, 1996. **153**(1): p. 75-81.
34. Hoffmann, J.A., et al., *Phylogenetic perspectives in innate immunity.* 1999. **284**(5418): p. 1313-1318.
35. Hashimoto, C., K.L. Hudson, and K.V.J.C. Anderson, *The Toll gene of Drosophila, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein.* 1988. **52**(2): p. 269-279.
36. Mahla, R.S., et al., *Sweeten PAMPs: role of sugar complexed PAMPs in innate immunity and vaccine biology.* 2013. **4**: p. 248.
37. Abbas, A.K., A.H. Lichtman, and S. Pillai, *Basic Immunology E-Book: Functions and Disorders of the Immune System*. 2019: Elsevier Health Sciences.
38. Di Lorenzo, A., et al., *Toll-Like Receptor 2 at the Crossroad between Cancer Cells, the Immune System, and the Microbiota.* 2020. **21**(24): p. 9418.
39. Kawai, T. and S.J.I.i. Akira, *The roles of TLRs, RLRs and NLRs in pathogen recognition.* 2009. **21**(4): p. 317-337.
40. Kumar, H., et al., *Toll-like receptors and innate immunity.* 2009. **388**(4): p. 621-625.
41. Medzhitov, R., P. Preston-Hurlburt, and C.A.J.N. Janeway, *A human homologue of the Drosophila Toll protein signals activation of adaptive immunity.* 1997. **388**(6640): p. 394-397.
42. Bell, J.K., et al., *The molecular structure of the Toll-like receptor 3 ligand-binding domain.* 2005. **102**(31): p. 10976-10980.
43. Heil, F., et al., *Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8.* 2004. **303**(5663): p. 1526-1529.
44. Barton, G.M., J.C. Kagan, and R.J.N.i. Medzhitov, *Intracellular localization of Toll-like receptor 9 prevents recognition of self DNA but facilitates access to viral DNA.* 2006. **7**(1): p. 49-56.
45. Belvin, M.P., K.V.J.A.r.o.c. Anderson, and d. biology, *A conserved signaling pathway: the Drosophila toll-dorsal pathway.* 1996. **12**(1): p. 393-416.
46. Latz, E., et al., *Ligand-induced conformational changes allosterically activate Toll-like receptor 9.* 2007. **8**(7): p. 772-779.
47. Jiang, Z., et al., *Details of Toll-like receptor: adapter interaction revealed by germ-line mutagenesis.* 2006. **103**(29): p. 10961-10966.
48. Dunne, A., et al., *Structural complementarity of Toll/interleukin-1 receptor domains in Toll-like receptors and the adaptors Mal and MyD88.* 2003. **278**(42): p. 41443-41451.
49. Li, C., J. Zienkiewicz, and J.J.J.o.B.C. Hawiger, *Interactive sites in the MyD88 Toll/interleukin (IL) 1 receptor domain responsible for coupling to the IL1β signaling pathway.* 2005. **280**(28): p. 26152-26159.
50. Hoebe, K., et al., *Identification of Lps2 as a key transducer of MyD88-independent TIR signalling.* 2003. **424**(6950): p. 743-748.
51. Ermolaeva, M.A., et al., *Function of TRADD in tumor necrosis factor receptor 1 signaling and in TRIF-dependent inflammatory responses.* 2008. **9**(9): p. 1037-1046.
52. Pobezinskaya, Y.L., et al., *The function of TRADD in signaling through tumor necrosis factor receptor 1 and TRIF-dependent Toll-like receptors.* 2008. **9**(9): p. 1047-1054.

53. Marshak-Rothstein, A.J.N.R.I., *Toll-like receptors in systemic autoimmune disease.* 2006. **6**(11): p. 823-835.
54. Ding, C., et al., *Plasmacytoid dendritic cells regulate autoreactive B cell activation via soluble factors and in a cell-to-cell contact manner.* 2009. **183**(11): p. 7140-7149.
55. Lentini, G., et al., *Neutrophils enhance their own influx to sites of bacterial infection via endosomal TLR-dependent Cxcl2 production.* 2020. **204**(3): p. 660-670.
56. Marshak-Rothstein, A., et al., *The stimulation of Toll-like receptors by nuclear antigens: a link between apoptosis and autoimmunity.* 2004. **30**(3): p. 559-574.
57. Parsa, R., et al., *BAFF-secreting neutrophils drive plasma cell responses during emergency granulopoiesis.* 2016. **213**(8): p. 1537-1553.
58. Giordano, D., et al., *B cell activating factor (BAFF) produced by neutrophils and dendritic cells is regulated differently and has distinct roles in Ab responses and protective immunity against West Nile virus.* 2020. **204**(6): p. 1508.
59. West, H.C. and C.L.J.F.i.i. Bennett, *Redefining the role of langerhans cells as immune regulators within the skin.* 2018. **8**: p. 1941.
60. Bevan, M.J.J.N.i., *Cross-priming.* 2006. **7**(4): p. 363-365.
61. Heath, W.R. and F.R.J.N.R.I. Carbone, *Cross-presentation in viral immunity and self-tolerance.* 2001. **1**(2): p. 126-134.
62. Schlitzer, A., et al., *Identification of cDC1-and cDC2-committed DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow.* 2015. **16**(7): p. 718-728.
63. Sieweke, M.H. and J.E.J.S. Allen, *Beyond stem cells: self-renewal of differentiated macrophages.* 2013. **342**(6161).
64. Kiessling, R., et al., *„Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell.* 1975. **5**(2): p. 117-121.
65. Kiessling, R., E. Klein, and H.J.E.j.o.i. Wigzell, *„Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype.* 1975. **5**(2): p. 112-117.
66. Herberman, R.B., M.E. Nunn, and D.H.J.I.j.o.c. Lavrin, *Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of reactivity and specificity.* 1975. **16**(2): p. 216-229.
67. Herberman, R.B., et al., *Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells.* 1975. **16**(2): p. 230-239.
68. Stokic-Trtica, V., A. Diefenbach, and C.S.J.F.i.I. Klose, *NK cell development in times of innate lymphoid cell diversity.* 2020. **11**.
69. Okamura, H., et al., *Regulation of interferon-γ production by IL-12 and IL-18.* 1998. **10**(3): p. 259-264.
70. Diefenbach, A., M. Colonna, and C.J.I. Romagnani, *The ILC world revisited.* 2017. **46**(3): p. 327-332.
71. Lauwerys, B.R., et al., *Cytokine production and killer activity of NK/T-NK cells derived with IL-2, IL-15, or the combination of IL-12 and IL-18.* 2000. **165**(4): p. 1847-1853.
72. Yawata, M., et al., *Roles for HLA and KIR polymorphisms in natural killer cell repertoire selection and modulation of effector function.* 2006. **203**(3): p. 633-645.
73. Kärre, K., et al., *Selective rejection of H–2-deficient lymphoma variants suggests alternative immune defence strategy.* 1986. **319**(6055): p. 675-678.
74. Cooper, M.A., T.A. Fehniger, and M.A.J.T.i.i. Caligiuri, *The biology of human natural killer-cell subsets.* 2001. **22**(11): p. 633-640.
75. Nielsen, N., et al., *Balance between activating NKG 2D, DNAM-1, NK p44 and NK p46 and inhibitory CD 94/NKG 2A receptors determine natural killer degranulation towards rheumatoid arthritis synovial fibroblasts.* 2014. **142**(4): p. 581-593.
76. Luckheeram, R.V., et al., *CD4+ T cells: differentiation and functions.* 2012. **2012**.
77. Mirshafiey, A., et al., *T-helper 22 cells as a new player in chronic inflammatory skin disorders.* 2015. **54**(8): p. 880-888.

78. Bettelli, E., et al., *Induction and effector functions of TH 17 cells.* 2008. **453**(7198): p. 1051-1057.
79. Romagnani, S.J.I.J.o.C. and L. Research, *Type 1 T helper and type 2 T helper cells: functions, regulation and role in protection and disease.* 1992. **21**(2-4): p. 152-158.
80. Mempel, T.R., et al., *Regulatory T cells reversibly suppress cytotoxic T cell function independent of effector differentiation.* 2006. **25**(1): p. 129-141.
81. Sakaguchi, S., et al., *Regulatory T cells: how do they suppress immune responses?* 2009. **21**(10): p. 1105-1111.
82. Fazilleau, N., et al., *Follicular helper T cells: lineage and location.* 2009. **30**(3): p. 324-335.
83. Nurieva, R.I., et al., *Bcl6 mediates the development of T follicular helper cells.* 2009. **325**(5943): p. 1001-1005.
84. Cooper, M.D., R.D. Peterson, and R.A.J.N. Good, *Delineation of the thymic and bursal lymphoid systems in the chicken.* 1965. **205**(4967): p. 143-146.
85. Cooper, M.D., et al., *The functions of the thymus system and the bursa system in the chicken.* 1966. **123**(1): p. 75-102.
86. Cambier, J.C., et al., *B-cell anergy: from transgenic models to naturally occurring anergic B cells?* 2007. **7**(8): p. 633-643.
87. Pieper, K., et al., *B-cell biology and development.* 2013. **131**(4): p. 959-971.
88. Davis, M.M. and P.J.J.N. Bjorkman, *T-cell antigen receptor genes and T-cell recognition.* 1988. **334**(6181): p. 395-402.
89. Zarnitsyna, V., et al., *Estimating the diversity, completeness, and cross-reactivity of the T cell repertoire.* 2013. **4**: p. 485.
90. Elhanati, Y., et al., *Inferring processes underlying B-cell repertoire diversity.* 2015. **370**(1676): p. 20140243.
91. Schatz, D.G. and Y.J.N.R.I. Ji, *Recombination centres and the orchestration of V (D) J recombination.* 2011. **11**(4): p. 251-263.
92. Ma, Y., et al., *Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V (D) J recombination.* 2002. **108**(6): p. 781-794.
93. Maynard, J. and G.J.A.r.o.b.e. Georgiou, *Antibody engineering.* 2000. **2**(1): p. 339-376.
94. Murphy, K. and C. Weaver, *Janeway's immunobiology*. 2016: Garland science.
95. Pillai, S. and A.J.N.R.I. Cariappa, *The follicular versus marginal zone B lymphocyte cell fate decision.* 2009. **9**(11): p. 767-777.
96. Allman, D. and S.J.C.o.i.i. Pillai, *Peripheral B cell subsets.* 2008. **20**(2): p. 149-157.
97. Attanavanich, K. and J.F.J.T.J.o.I. Kearney, *Marginal zone, but not follicular B cells, are potent activators of naive CD4 T cells.* 2004. **172**(2): p. 803-811.
98. Kraal, G. and M.J.I. Janse, *Marginal metallophilic cells of the mouse spleen identified by a monoclonal antibody.* 1986. **58**(4): p. 665.
99. Cinamon, G., et al., *Follicular shuttling of marginal zone B cells facilitates antigen transport.* 2008. **9**(1): p. 54-62.
100. Ferguson, A.R., M.E. Youd, and R.B.J.I.i. Corley, *Marginal zone B cells transport and deposit IgM-containing immune complexes onto follicular dendritic cells.* 2004. **16**(10): p. 1411-1422.
101. Arnon, T.I., et al., *Visualization of splenic marginal zone B-cell shuttling and follicular B-cell egress.* 2013. **493**(7434): p. 684-688.
102. Pillai, S., A. Cariappa, and S.T.J.A.R.I. Moran, *Marginal zone B cells.* 2005. **23**: p. 161-196.
103. Grönwall, C., J. Vas, and G.J.J.F.i.i. Silverman, *Protective roles of natural IgM antibodies.* 2012. **3**: p. 66.
104. Evans, J.G., et al., *Novel suppressive function of transitional 2 B cells in experimental arthritis.* 2007. **178**(12): p. 7868-7878.
105. Gray, M., et al., *Apoptotic cells protect mice from autoimmune inflammation by the induction of regulatory B cells.* 2007. **104**(35): p. 14080-14085.

106. Spencer, N. and R.A.J.I.i. Daynes, *IL-12 directly stimulates expression of IL-10 by CD5+ B cells and IL-6 by both CD5+ and CD5-B cells: possible involvement in age-associated cytokine dysregulation.* 1997. **9**(5): p. 745-754.
107. Fillatreau, S., et al., *B cells regulate autoimmunity by provision of IL-10.* 2002. **3**(10): p. 944-950.
108. Lampropoulou, V., et al., *TLR-activated B cells suppress T cell-mediated autoimmunity.* 2008. **180**(7): p. 4763-4773.
109. Imbrechts, M., et al., *IFN-γ stimulates CpG-induced IL-10 production in B cells via p38 and JNK signalling pathways.* 2018. **48**(9): p. 1506-1521.
110. Sattler, S., et al., *IL-10-producing regulatory B cells induced by IL-33 (BregIL-33) effectively attenuate mucosal inflammatory responses in the gut.* 2014. **50**: p. 107-122.
111. Hua, C., et al., *A proliferation inducing ligand (APRIL) promotes IL-10 production and regulatory functions of human B cells.* 2016. **73**: p. 64-72.
112. Rosser, E.C., et al., *Regulatory B cells are induced by gut microbiota–driven interleukin-1β and interleukin-6 production.* 2014. **20**(11): p. 1334-1339.
113. Hoffman, W., F.G. Lakkis, and G.J.C.J.o.t.A.S.o.N. Chalasani, *B cells, antibodies, and more.* 2016. **11**(1): p. 137-154.
114. Chen, X. and P.E.J.A.i.e.t.e. Jensen, *The role of B lymphocytes as antigen-presenting cells.* 2008. **56**(2): p. 77.
115. Malynn, B., D. Romeo, and H.J.T.J.o.I. Wortis, *Antigen-specific B cells efficiently present low doses of antigen for induction of T cell proliferation.* 1985. **135**(2): p. 980-988.
116. Lund, F.E. and T.D.J.N.R.I. Randall, *Effector and regulatory B cells: modulators of CD4+ T cell immunity.* 2010. **10**(4): p. 236-247.
117. León, B., et al., *Unraveling effector functions of B cells during infection: the hidden world beyond antibody production.* 2012. **12**(3): p. 213-221.
118. Crawford, A., et al., *Primary T cell expansion and differentiation in vivo requires antigen presentation by B cells.* 2006. **176**(6): p. 3498-3506.
119. Cheng, A.M., et al., *Syk tyrosine kinase required for mouse viability and B-cell development.* 1995. **378**(6554): p. 303-306.
120. Kurosaki, T., et al., *Syk activation by the Src-family tyrosine kinase in the B cell receptor signaling.* 1994. **179**(5): p. 1725-1729.
121. Rickert, R.C.J.C.o.i.i., *Regulation of B lymphocyte activation by complement C3 and the B cell coreceptor complex.* 2005. **17**(3): p. 237-243.
122. Susa, K.J., et al., *A dynamic interaction between CD19 and the tetraspanin CD81 controls B cell co-receptor trafficking.* 2020. **9**: p. e52337.
123. Ohl, L., et al., *Cooperating mechanisms of CXCR5 and CCR7 in development and organization of secondary lymphoid organs.* 2003. **197**(9): p. 1199-1204.
124. Hardtke, S., L. Ohl, and R.J.B. Förster, *Balanced expression of CXCR5 and CCR7 on follicular T helper cells determines their transient positioning to lymph node follicles and is essential for efficient B-cell help.* 2005. **106**(6): p. 1924-1931.
125. Hasbold, J., et al., *Integrating signals from IFN-γ and IL-4 by B cells: positive and negative effects on CD40 ligand-induced proliferation, survival, and division-linked isotype switching to IgG1, IgE, and IgG2a.* 1999. **163**(8): p. 4175-4181.
126. Gauchat, J.-F., et al., *Modulation of IL-4 induced germline ε RNA synthesis in human B cells by tumor necrosis factor-α, anti-CD40 monoclonal antibodies or transforming growth factor-β correlates with levels of IgE production.* 1992. **4**(3): p. 397-406.
127. Hutloff, A., et al., *ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28.* 1999. **397**(6716): p. 263-266.
128. MacLennan, I.J.C.B., *Somatic mutation: from the dark zone to the light.* 1994. **4**(1): p. 70-72.
129. Victora, G.D., et al., *Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter.* 2010. **143**(4): p. 592-605.

130. Allen, C.D., T. Okada, and J.G.J.I. Cyster, *Germinal-center organization and cellular dynamics.* 2007. **27**(2): p. 190-202.
131. Stebegg, M., et al., *Regulation of the germinal center response.* 2018. **9**: p. 2469.
132. Wabl, M. and C.J.C.o.i.i. Steinberg, *Affinity maturation and class switching.* 1996. **8**(1): p. 89-92.
133. Arakawa, H., J. Hauschild, and J.-M.J.S. Buerstedde, *Requirement of the activation-induced deaminase (AID) gene for immunoglobulin gene conversion.* 2002. **295**(5558): p. 1301-1306.
134. MacLennan, I.C.J.A.r.o.i., *Germinal centers.* 1994. **12**(1): p. 117-139.
135. Muramatsu, M., et al., *Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells.* 1999. **274**(26): p. 18470-18476.
136. Muramatsu, M., et al., *Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme.* 2000. **102**(5): p. 553-563.
137. JV, R.J.C., *Siebenlist U. Korsmeyer S. Waldmann T. Leder P. Structure of the human immunoglobulin m locus: characterization of embryonic and rearranged J and D genes.* 1981. **27**(583-591): p. 11421.
138. Mizushima, N. and D.J.J.A.R.N. Klionsky, *Protein turnover via autophagy: implications for metabolism.* 2007. **27**: p. 19-40.
139. Mizushima, N., et al., *Autophagosome formation in mammalian cells.* 2002. **27**(6): p. 421-429.
140. Massey, A.C., C. Zhang, and A.M.J.C.t.i.d.b. Cuervo, *Chaperone-mediated autophagy in aging and disease.* 2006. **73**: p. 205-235.
141. Johansen, T. and T.J.A. Lamark, *Selective autophagy mediated by autophagic adapter proteins.* 2011. **7**(3): p. 279-296.
142. Mizushima, N., et al., *A protein conjugation system essential for autophagy.* 1998. **395**(6700): p. 395-398.
143. Ohsumi, Y.J.N.r.M.c.b., *Molecular dissection of autophagy: two ubiquitin-like systems.* 2001. **2**(3): p. 211-216.
144. Levine, B. and V.J.N.R.I. Deretic, *Unveiling the roles of autophagy in innate and adaptive immunity.* 2007. **7**(10): p. 767-777.
145. Miller, B.C., et al., *The autophagy gene ATG5 plays an essential role in B lymphocyte development.* 2008. **4**(3): p. 309-314.
146. Conway, K.L., et al., *ATG5 regulates plasma cell differentiation.* 2013. **9**(4): p. 528-537.
147. Arnold, J., et al., *Autophagy is dispensable for B-cell development but essential for humoral autoimmune responses.* 2016. **23**(5): p. 853-864.
148. Martinez-Martin, N., et al., *A switch from canonical to noncanonical autophagy shapes B cell responses.* 2017. **355**(6325): p. 641-647.
149. Anand, P.K., et al., *TLR2 and RIP2 pathways mediate autophagy of Listeria monocytogenes via extracellular signal-regulated kinase (ERK) activation.* 2011. **286**(50): p. 42981-42991.
150. Travassos, L.H., et al., *Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry.* 2010. **11**(1): p. 55-62.
151. Salio, M., et al., *Biology of CD1-and MR1-restricted T cells.* 2014. **32**: p. 323-366.
152. Bendelac, A., et al., *A subset of CD4+ thymocytes selected by MHC class I molecules.* 1994. **263**(5154): p. 1774-1778.
153. Bendelac, A., et al., *CD1 recognition by mouse NK1+ T lymphocytes.* 1995. **268**(5212): p. 863-865.
154. Jahng, A., et al., *Prevention of autoimmunity by targeting a distinct, noninvariant CD1d-reactive T cell population reactive to sulfatide.* 2004. **199**(7): p. 947-957.
155. Leadbetter, E.A., et al., *NK T cells provide lipid antigen-specific cognate help for B cells.* 2008. **105**(24): p. 8339-8344.
156. Cerundolo, V., et al., *Harnessing invariant NKT cells in vaccination strategies.* 2009. **9**(1): p. 28-38.
157. Niemeyer, M., et al., *Natural killer T-cell characterization through gene expression profiling: an account of versatility bridging T helper type 1 (Th1), Th2 and Th17 immune responses.* 2008. **123**(1): p. 45-56.

158. Clerici, L., G. Casorati, and P.J.A. Dellabona, *B Cell Help by CD1d-Rectricted NKT Cells.* 2015. **4**(4): p. 279-294.
159. Barral, P., et al., *B cell receptor-mediated uptake of CD1d-restricted antigen augments antibody responses by recruiting invariant NKT cell help in vivo.* 2008. **105**(24): p. 8345-8350.
160. Chang, P.-P., et al., *Identification of Bcl-6-dependent follicular helper NKT cells that provide cognate help for B cell responses.* 2012. **13**(1): p. 35-43.
161. Sedimbi, S.K., et al., *Combined proinflammatory cytokine and cognate activation of invariant natural killer T cells enhances anti-DNA antibody responses.* 2020. **117**(16): p. 9054-9063.
162. Barral, P., et al., *The location of splenic NKT cells favours their rapid activation by blood-borne antigen.* 2012. **31**(10): p. 2378-2390.
163. King, I.L., et al., *The mechanism of splenic invariant NKT cell activation dictates localization in vivo.* 2013. **191**(2): p. 572-582.
164. Devera, G.A.L., et al., *BAFF-and APRIL-Dependent Maintenance.* 2013.
165. Tonti, E., et al., *Follicular helper NKT cells induce limited B cell responses and germinal center formation in the absence of CD4+ T cell help.* 2012. **188**(7): p. 3217-3222.
166. Vinuesa, C.G., et al., *Follicular B helper T cells in antibody responses and autoimmunity.* 2005. **5**(11): p. 853-865.
167. Detre, C., et al., *SAP expression in invariant NKT cells is required for cognate help to support B-cell responses.* 2012. **120**(1): p. 122-129.
168. King, I.L., et al., *Invariant natural killer T cells direct B cell responses to cognate lipid antigen in an IL-21-dependent manner.* 2012. **13**(1): p. 44.
169. Wilson, M.T., et al., *The response of natural killer T cells to glycolipid antigens is characterized by surface receptor down-modulation and expansion.* 2003. **100**(19): p. 10913-10918.
170. Harada, M., et al., *Down-regulation of the invariant Vα14 antigen receptor in NKT cells upon activation.* 2004. **16**(2): p. 241-247.
171. Crowe, N.Y., et al., *Glycolipid antigen drives rapid expansion and sustained cytokine production by NK T cells.* 2003. **171**(8): p. 4020-4027.
172. Parekh, V.V., et al., *Glycolipid antigen induces long-term natural killer T cell anergy in mice.* 2005. **115**(9): p. 2572-2583.
173. Schwartz, R.H.J.A.r.o.i., *T cell anergy.* 2003. **21**(1): p. 305-334.
174. Yamagami, T., et al., *Frequencies of Multiple IgL Chain Gene Rearrangements in Single Normal or κL Chain–Deficient B Lineage Cells.* 1999. **11**(3): p. 317-327.
175. Von Boehmer, H., et al., *Thymic selection revisited: how essential is it?* 2003. **191**(1): p. 62-78.
176. ten Boekel, E., F. Melchers, and A.G.J.I. Rolink, *Changes in the VH gene repertoire of developing precursor B lymphocytes in mouse bone marrow mediated by the pre-B cell receptor.* 1997. **7**(3): p. 357-368.
177. Von Boehmer, H. and F.J.N.i. Melchers, *Checkpoints in lymphocyte development and autoimmune disease.* 2010. **11**(1): p. 14.
178. von Boehmer, H.J.A.i.i., *Selection of the T-cell repertoire: receptor-controlled checkpoints in T-cell development.* 2004. **84**: p. 201-238.
179. Wardemann, H. and M.C.J.A.i.i. Nussenzweig, *B-cell self-tolerance in humans.* 2007. **95**: p. 83-110.
180. Feng, Y., et al., *A mechanism for expansion of regulatory T-cell repertoire and its role in self-tolerance.* 2015. **528**(7580): p. 132-136.
181. Pasare, C. and R.J.S. Medzhitov, *Toll pathway-dependent blockade of CD4+ CD25+ T cell-mediated suppression by dendritic cells.* 2003. **299**(5609): p. 1033-1036.
182. Haile, L.A., T.F. Greten, and F.J.I.i. Korangy, *Immune suppression: the hallmark of myeloid derived suppressor cells.* 2012. **41**(6-7): p. 581-594.
183. Li, M.O. and A.Y.J.N.R.I. Rudensky, *T cell receptor signalling in the control of regulatory T cell differentiation and function.* 2016. **16**(4): p. 220-233.

184. Scalapino, K.J. and D.I.J.I.r. Daikh, *CTLA-4: a key regulatory point in the control of autoimmune disease.* 2008. **223**(1): p. 143-155.
185. Kunz, M. and S.M.J.M.o.i. Ibrahim, *Cytokines and cytokine profiles in human autoimmune diseases and animal models of autoimmunity.* 2009. **2009**.
186. Sakaguchi, S.J.A.R.I., *Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses.* 2004. **22**: p. 531-562.
187. Tao, J.-H., et al., *Foxp3, regulatory T cell, and autoimmune diseases.* 2017. **40**(1): p. 328-339.
188. Gorantla, V.S., et al., *T regulatory cells and transplantation tolerance.* 2010. **24**(3): p. 147-159.
189. Issa, F., A. Schiopu, and K.J.J.E.r.o.c.i. Wood, *Role of T cells in graft rejection and transplantation tolerance.* 2010. **6**(1): p. 155-169.
190. Lu, L., H.J.C. Cantor, and m. immunology, *Generation and regulation of CD8+ regulatory T cells.* 2008. **5**(6): p. 401-406.
191. Kapp, J.A. and R.P.J.H.i. Bucy, *CD8+ suppressor T cells resurrected.* 2008. **69**(11): p. 715-720.
192. Yamagata, T., D. Mathis, and C.J.N.i. Benoist, *Self-reactivity in thymic double-positive cells commits cells to a CD8αα lineage with characteristics of innate immune cells.* 2004. **5**(6): p. 597-605.
193. Hu, D., et al., *Analysis of regulatory CD8 T cells in Qa-1-deficient mice.* 2004. **5**(5): p. 516-523.
194. Jiang, H., et al., *HLA-E–restricted regulatory CD8+ T cells are involved in development and control of human autoimmune type 1 diabetes.* 2010. **120**(10): p. 3641-3650.
195. Liu, J., et al., *Rat CD8+ FOXP3+ T suppressor cells mediate tolerance to allogeneic heart transplants, inducing PIR-B in APC and rendering the graft invulnerable to rejection.* 2004. **13**(4): p. 239-247.
196. Huber, M., et al., *IL-17A secretion by CD8+ T cells supports Th17-mediated autoimmune encephalomyelitis.* 2012. **123**(1).
197. Giles, J.R., et al., *Autoreactive helper T cells alleviate the need for intrinsic TLR signaling in autoreactive B cell activation.* 2017. **2**(4).
198. Carter, N.A., et al., *Mice lacking endogenous IL-10–producing regulatory B cells develop exacerbated disease and present with an increased frequency of Th1/Th17 but a decrease in regulatory T cells.* 2011. **186**(10): p. 5569-5579.
199. Knippenberg, S., et al., *Reduction in IL-10 producing B cells (Breg) in multiple sclerosis is accompanied by a reduced naive/memory Breg ratio during a relapse but not in remission.* 2011. **239**(1-2): p. 80-86.
200. Mielle, J., et al., *IL-10 producing B cells ability to induce regulatory T cells is maintained in rheumatoid arthritis.* 2018. **9**: p. 961.
201. Flores-Borja, F., et al., *CD19+ CD24hiCD38hi B cells maintain regulatory T cells while limiting TH1 and TH17 differentiation.* 2013. **5**(173): p. 173ra23-173ra23.
202. Gray, M. and D.J.I.i. Gray, *Regulatory B cells mediate tolerance to apoptotic self in health: implications for disease.* 2015. **27**(10): p. 505-511.
203. Miles, K., et al., *Immune tolerance to apoptotic self is mediated primarily by regulatory B1a cells.* 2018. **8**: p. 1952.
204. Jin, L.P., D.X. Fan, and D.J.J.A.J.o.R.I. Li, *Regulation of costimulatory signal in maternal–fetal immune tolerance.* 2011. **66**(2): p. 76-83.
205. Masi, A.T., R.A.J.A. Kaslow, and R.O.J.o.t.A.C.o. Rheumatology, *Sex effects in systemic lupus erythematosus. A clue to pathogenesis.* 1978. **21**(4): p. 480-484.
206. Fernández, M., et al., *A multiethnic, multicenter cohort of patients with systemic lupus erythematosus (SLE) as a model for the study of ethnic disparities in SLE.* 2007. **57**(4): p. 576-584.
207. Deafen, D., et al., *A revised estimate of twin concordance in systemic lupus erythematosus.* 1992. **35**(3): p. 311-318.
208. Marquart, H., et al., *Complement receptor expression and activation of the complement cascade on B lymphocytes from patients with systemic lupus erythematosus (SLE).* 1995. **101**(1): p. 60-65.
209. Sturfelt, G. and L.J.R. Truedsson, *Complement and its breakdown products in SLE.* 2005. **44**(10): p. 1227-1232.

210. Pradhan, V.D., et al., *Anti-neutrophil cytoplasmic antibodies (ANCA) in systemic lupus erythematosus: prevalence, clinical associations and correlation with other autoantibodies.* 2004. **52**: p. 533-537.
211. Dixon, F.J., et al., *Etiology and pathogenesis of a spontaneous lupus-like syndrome in mice.* 1978. **21**(S1): p. S64-S67.
212. Rauch, J., et al., *A high frequency idiotypic marker of anti-DNA autoantibodies in MRL-Ipr/Ipr mice.* 1982. **129**(1): p. 236-241.
213. Steinberg, A.D., et al., *Effects of thymectomy or androgen administration upon the autoimmune disease of MRL/Mp-lpr/lpr mice.* 1980. **125**(2): p. 871-873.
214. Pisetsky, D., et al., *Ipr gene control of the anti-DNA antibody response.* 1982. **128**(5): p. 2322-2325.
215. Wu, J., et al., *Autoimmune disease in mice due to integration of an endogenous retrovirus in an apoptosis gene.* 1993. **178**(2): p. 461-468.
216. Mountz, J.D., et al., *Apoptosis defects analyzed in TcR transgenic and fas transgenic Ipr mice.* 1994. **11**(4): p. 321-342.
217. Mountz, J., et al., *Autoimmune disease results from multiple interactive defects in apoptosis induction molecules and signaling pathways.* 1996(97): p. 200-219.
218. Alexander, E.L., et al., *Congenic autoimmune murine models of central nervous system diseas in connective tissue disorders.* 1983. **14**(2): p. 242-248.
219. Eisenberg, R., E. Tan, and F.J.T.J.o.e.m. Dixon, *Presence of anti-Sm reactivity in autoimmune mouse strains.* 1978. **147**(2): p. 582-587.
220. Alexander, E.L., et al., *Two histopathologic types of inflammatory vascular disease in MRL/Mp autoimmune mice. Model for human vasculitis in connective tissue disease.* 1985. **28**(10): p. 1146-1155.
221. Satoh, M. and W.H.J.T.J.o.e.m. Reeves, *Induction of lupus-associated autoantibodies in BALB/c mice by intraperitoneal injection of pristane.* 1994. **180**(6): p. 2341-2346.
222. Yokogawa, M., et al., *Epicutaneous application of Toll-like receptor 7 agonists leads to systemic autoimmunity in wild-type mice: a new model of systemic lupus erythematosus.* 2014. **66**(3): p. 694-706.
223. Yoshida, H., et al., *Effect of an exogenous trigger on the pathogenesis of lupus in (NZB× NZW) F1 mice.* 2002. **46**(8): p. 2235-2244.
224. Mevorach, D., et al., *Systemic exposure to irradiated apoptotic cells induces autoantibody production.* 1998. **188**(2): p. 387-392.
225. Duhlin, A., et al., *Selective memory to apoptotic cell–derived self-antigens with implications for systemic lupus erythematosus development.* 2016. **197**(7): p. 2618-2626.
226. Holodick, N.E., et al., *Expansion of B-1a cells with germline heavy chain sequence in lupus mice.* 2016. **7**: p. 108.
227. Viau, M. and M.J.C.I. Zouali, *B-lymphocytes, innate immunity, and autoimmunity.* 2005. **114**(1): p. 17-26.
228. Cyster, J.G.J.S., *Chemokines and cell migration in secondary lymphoid organs.* 1999. **286**(5447): p. 2098-2102.
229. Pisetsky, D.S.J.N.R.R., *Antinuclear antibody testing—misunderstood or misbegotten?* 2017. **13**(8): p. 495.
230. Odendahl, M., et al., *Generation of migratory antigen-specific plasma blasts and mobilization of resident plasma cells in a secondary immune response.* 2005. **105**(4): p. 1614-1621.
231. Quiding-Järbrink, M., et al., *Differential expression of tissue-specific adhesion molecules on human circulating antibody-forming cells after systemic, enteric, and nasal immunizations. A molecular basis for the compartmentalization of effector B cell responses.* 1997. **99**(6): p. 1281-1286.
232. Quiding-Järbrink, M., et al., *Human circulating specific antibody-forming cells after systemic and mucosal immunizations: differential homing commitments and cell surface differentiation markers.* 1995. **25**(2): p. 322-327.
233. Muehlinghaus, G., et al., *Regulation of CXCR3 and CXCR4 expression during terminal differentiation of memory B cells into plasma cells.* 2005. **105**(10): p. 3965-3971.

234. Ferraro, A.J., et al., *Levels of autoantibodies, unlike antibodies to all extrinsic antigen groups, fall following B cell depletion with Rituximab.* 2008. **38**(1): p. 292-298.
235. Hoyer, B.F., et al. *Short-lived plasmablasts and long-lived plasma cells contribute to chronic humoral autoimmunity in NZB/W mice*. in *Arthritis Res Ther*. 2004. Springer.
236. Kehrl, J.H., et al., *Molecular mechanisms regulating CD19, CD20 and CD22 gene expression.* 1994. **15**(9): p. 432-436.
237. Leandro, M.J.J.A.r. and therapy, *B-cell subpopulations in humans and their differential susceptibility to depletionwith anti-CD20 monoclonal antibodies.* 2013. **15**(1): p. 1-8.
238. Fabris, M., et al., *Serum levels of anti-CCP antibodies, anti-MCV antibodies and RF IgA in the follow-up of patients with rheumatoid arthritis treated with rituximab.* 2010. **1**(2): p. 87-94.
239. Agematsu, K., et al., *Generation of plasma cells from peripheral blood memory B cells: synergistic effect of interleukin-10 and CD27/CD70 interaction.* 1998. **91**(1): p. 173-180.
240. Muto, K., et al., *Memory B cell resurgence requires repeated rituximab in myasthenia gravis.* 2017. **27**(10): p. 918-922.
241. Reddy, V., et al., *Obinutuzumab induces superior B-cell cytotoxicity to rituximab in rheumatoid arthritis and systemic lupus erythematosus patient samples.* 2017. **56**(7): p. 1227-1237.
242. Tedder, T.F. and P.J.I.t. Engel, *CD20: a regulator of cell-cycle progression of B lymphocytes.* 1994. **15**(9): p. 450-454.
243. Ng, K.P., et al., *B cell depletion therapy in systemic lupus erythematosus: long-term follow-up and predictors of response.* 2007. **66**(9): p. 1259-1262.
244. Tedder, T.F., L.-J. Zhou, and P.J.I.t. Engel, *The CD19/CD21 signal transduction complex of B lymphocytes.* 1994. **15**(9): p. 437-442.
245. Yazawa, N., et al., *Immunotherapy using unconjugated CD19 monoclonal antibodies in animal models for B lymphocyte malignancies and autoimmune disease.* 2005. **102**(42): p. 15178-15183.
246. Hamaguchi, Y., et al., *The peritoneal cavity provides a protective niche for B1 and conventional B lymphocytes during anti-CD20 immunotherapy in mice.* 2005. **174**(7): p. 4389-4399.
247. Moore, P.A., et al., *BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator.* 1999. **285**(5425): p. 260-263.
248. Schneider, P., et al., *BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth.* 1999. **189**(11): p. 1747-1756.
249. Thien, M., et al., *Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches.* 2004. **20**(6): p. 785-798.
250. Baker, K.P., et al., *Generation and characterization of LymphoStat-B, a human monoclonal antibody that antagonizes the bioactivities of B lymphocyte stimulator.* 2003. **48**(11): p. 3253-3265.
251. Dooley, M., et al., *Effect of belimumab treatment on renal outcomes: results from the phase 3 belimumab clinical trials in patients with SLE.* 2013. **22**(1): p. 63-72.
252. Bishop, G.A., B.S.J.C. Hostager, and g.f. reviews, *The CD40–CD154 interaction in B cell–T cell liaisons.* 2003. **14**(3-4): p. 297-309.
253. Kalunian, K.C., et al., *Treatment of systemic lupus erythematosus by inhibition of T cell costimulation with anti-CD154: a randomized, double-blind, placebo-controlled trial.* 2002. **46**(12): p. 3251-3258.
254. Boumpas, D.T., et al., *A short course of BG9588 (anti–CD40 ligand antibody) improves serologic activity and decreases hematuria in patients with proliferative lupus glomerulonephritis.* 2003. **48**(3): p. 719-727.
255. Marken, J., et al., *Anti-CD40 antibody KPL-404 inhibits T cell-mediated activation of B cells from healthy donors and autoimmune patients.* 2021. **23**(1): p. 1-15.
256. Hammond, K.J. and M.J.C.o.i.i. Kronenberg, *Natural killer T cells: natural or unnatural regulators of autoimmunity?* 2003. **15**(6): p. 683-689.
257. Sireci, G., et al., *Immunoregulatory role of Jα281 T cells in aged mice developing lupus-like nephritis.* 2007. **37**(2): p. 425-433.
258. Bach, J.F.J.N.R.I., *Regulatory T cells under scrutiny.* 2003. **3**(3): p. 189-198.

259. Hägglöf, T., et al., *Neutrophils license i NKT cells to regulate self-reactive mouse B cell responses.* 2016. **17**(12): p. 1407-1414.
260. Oleinika, K., et al., *CD1d-dependent immune suppression mediated by regulatory B cells through modulations of iNKT cells.* 2018. **9**(1): p. 1-17.
261. Baglaenko, Y., et al., *Suppression of autoimmunity by CD5+ IL-10-producing B cells in lupus-prone mice.* 2015. **16**(5): p. 311-320.
262. Baglaenko, Y., et al., *IL-10 production is critical for sustaining the expansion of CD5+ B and NKT cells and restraining autoantibody production in congenic lupus-prone mice.* 2016. **11**(3): p. e0150515.
263. Yang, J.-Q., et al., *Invariant NKT cells inhibit autoreactive B cells in a contact-and CD1d-dependent manner.* 2011. **186**(3): p. 1512-1520.
264. Wermeling, F., et al., *Invariant NKT cells limit activation of autoreactive CD1d-positive B cells.* 2010. **207**(5): p. 943-952.
265. Nakamura, M., et al., *High frequency class switching of an lgM+ B lymphoma clone CH12F3 to lgA+ cells.* 1996. **8**(2): p. 193-201.
266. Kashiwamura, S., et al., *Structure of the murine mb-1 gene encoding a putative sIgM-associated molecule.* Journal of immunology (Baltimore, Md. : 1950), 1990. **145**(1): p. 337.
267. Cifarelli, V., et al., *CD36 Deficiency Impairs the Small Intestinal Barrier and Induces Subclinical Inflammation in Mice.* Cellular and Molecular Gastroenterology and Hepatology, 2017. **3**(1): p. 82-98.
268. Robbiani, D.F., et al., *AID is required for the chromosomal breaks in c-myc that lead to c-myc/IgH translocations.* 2008. **135**(6): p. 1028-1038.
269. Christensen, S.R., et al., *Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus.* 2006. **25**(3): p. 417-428.
270. Nutt, S.L., et al., *The generation of antibody-secreting plasma cells.* 2015. **15**(3): p. 160-171.
271. Cancro, M.P.J.A.r.o.i., *Age-associated B cells.* 2020. **38**: p. 315-340.
272. Jackson, S.W., et al., *Opposing impact of B cell–intrinsic TLR7 and TLR9 signals on autoantibody repertoire and systemic inflammation.* 2014. **192**(10): p. 4525-4532.