Dysregulation of autoreactive B cell responses in autoimmune diseases: from initial triggering to persistent activation

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1

CHAPTER 1

Introduction on autoreactive B cell responses in autoimmune diseases

B cell responses: from protective to pathogenic

B cells represent an important component of the human immune system by playing a crucial role in the defense against microbes. B cells exert their function through multiple effector mechanisms, including the secretion of protective antibodies (Fig. 1). B cells have the ability to respond to a wide variety of microbes, to adapt to the pathogen and to provide long-lasting immunity. The response of B cells to microbes is tightly regulated to ensure an appropriate response while minimizing unwarranted side effects.

A loss in the tight regulation of B cells can lead to the generation of autoreactive B cell responses that are directed against structures of the own body (Fig. 1). The presence of autoreactive B cell responses is a hallmark of many autoimmune diseases and autoreactive B cells are thought to play an important role in the pathogenesis of these diseases. However, both the biology underlying the disturbed regulation of autoreactive B cell responses and their exact role in autoimmune diseases are poorly understood.

To investigate how processes regulating B cells are altered in autoimmune diseases, it is crucial to understand the fundamental processes underlying protective B cell immunity. Therefore, this chapter provides an introduction on general concepts of B cell biology, an overview of the current state of knowledge on the role of autoreactive B cells in autoimmune diseases, and ultimately an outline of the research presented in this thesis.

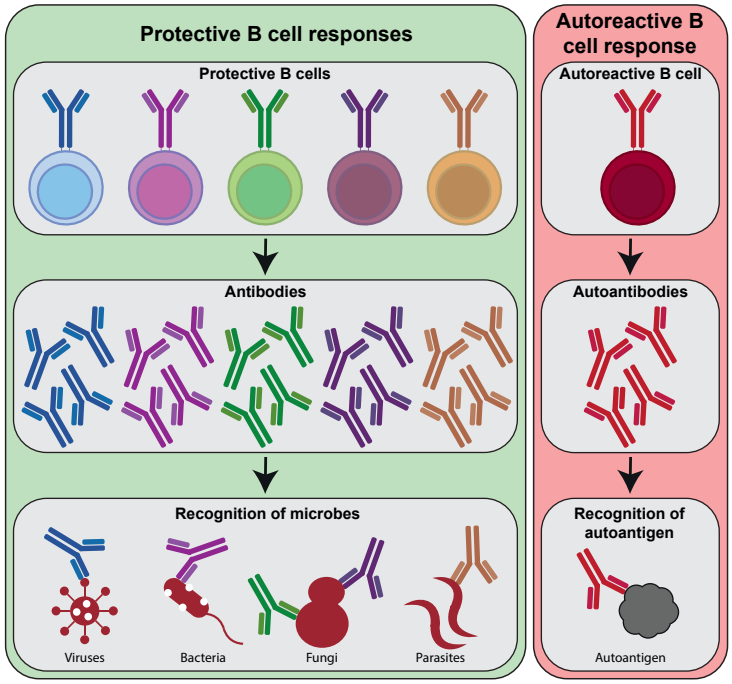

Figure 1. B cell responses: from protective to pathogenic. Protective B cells responses provide a broad layer of protection against microbes by the generation of antibodies. In contrast, autoreactive B cell responses are directed against structures of the own body (autoantigens) and are a hallmark of many autoimmune diseases.

Cornerstone of B cell biology: B cell repertoire

B cells can recognize microbes based on their expression of membrane-bound antibody, termed the B cell receptor (BCR). Each B cell has a unique BCR that can bind to a highly specific structure, termed the antigen. Together, the large pool of B cells in the human body forms a diverse repertoire which governs reactivity against a wide spectrum of antigens (Fig. 1)[1,2].

The variability in antigen specificity of antibodies, either in secreted form or expressed on the membrane of B cells, resides in a specific part of their structure. Antibodies are Y-shaped molecules composed of two identical structures consisting of a heavy chain (HC) and a light chain (LC) which are connected by disulfide bridges (Fig. 2)[3]. These HCs and LCs have a variable domain and a constant domain. While the constant domain is similar between antibodies, the variable domain is highly unique and determines antigen specificity. The variable domain of the HC consists of a variable (V), diversity (D) and joining (J) segment, while the LC only has a V and a J segment. These V, D and J segments are assembled during B cell development by genetic recombination from a broad set of V, D and J segments which are encoded in the human genome[4]. This random recombination process in combination with pairing of HC and LC can, in theory, form billions of unique antibodies[5]. In later stages of B cell differentiation, the antibody structure can be further modified by acquisition of somatic mutations in the variable domain[6]. Together, these processes are critical in the formation of a diverse BCR repertoire capable of fighting a broad range of pathogens.

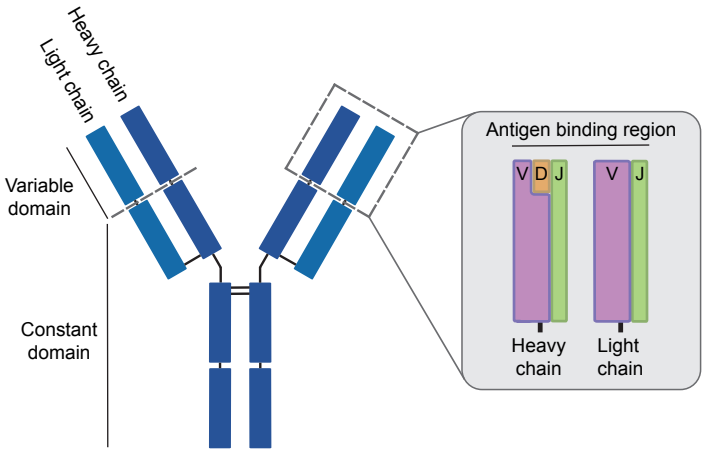

Figure 2. Schematic representation of an antibody. Antibodies are Y-shaped molecules containing two heavy chains (HC) and two light chains (LC). Antibodies have a constant domain and a variable domain. The variable domain contains the antigen binding regions and consists of a V, D and J segment in the heavy chain and a V and J segment in the light chain.

B cell development

The development of B cells is tailored to generate a diverse pool of B cells, while preventing the generation of B cells with dysfunctional BCRs or autoreactive BCRs that can recognize self-antigens. B cell development takes place in the bone marrow and is

initiated by the commitment of a hematopoietic stem cell progenitor to the B cell lineage by differentiating into a pro-B cell (Fig. 3)[7,8]. These pro-B cells undergo the first step in acquiring a BCR by the formation of a HC through genetic rearrangement and joining of V, D and J segments[9]. When this rearrangement succeeds and the HC is expressed in complex with a surrogate light chain, pro-B cells become pre-B cells[10].

Pre-B cells expressing a functional HC will proliferate, subsequently exit the cell cycle and start with the formation of a LC[11]. Gene rearrangement of the LC only involves rearrangement of a V and a J gene segment and initially takes place at a kappa locus. If the LC can be functionally paired with the HC, immature B cells can express a complete BCR on their surface and are able to sense antigen[12]. The expression of a functional BCR by immature B cells induces maturation and survival of the cell[13]. Ultimately, immature B cells differentiate into transitional cells which migrate from the bone marrow to the circulation[14]. In the periphery, B cells finalize their maturation to become naïve B cells.

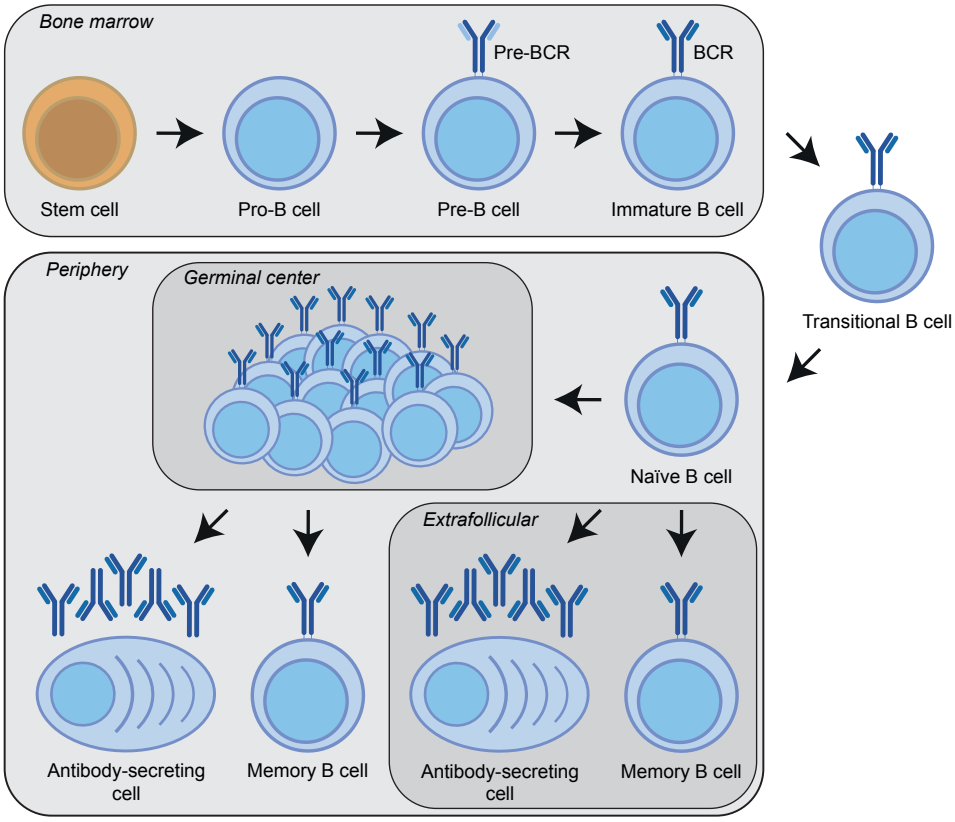

Figure 3. Overview of B cell development and B cell differentiation. B cell development takes place in the bone marrow where B cells differentiate from a stem cell to an immature B cell with a B cell receptor. Transitional B cells leave the bone marrow and complete their maturation to become naïve B cells in the periphery. Naïve B cells can be activated in the periphery upon encountering antigen. Activated B cells can, upon interaction with T cells, differentiate through an extrafollicular or a germinal center pathway. Boths pathways generate memory B cells and antibody-secreting cells.

Central tolerance

The formation of BCRs by V(D)J recombination during B cell development is the result of random processes. This randomness is important for the generation of a diverse BCR repertoire, but also leads to the formation of autoreactive BCRs. In fact, the majority of immature B cells generated in the bone marrow are thought to be autoreactive[15,16]. There are multiple mechanisms, collectively referred to as central tolerance, that prevent these autoreactive B cells from ending up in the circulation and potentially initiating an immune reaction against the own body[17].

Receptor editing is an important central tolerance mechanism in which B cells attempt to change the specificity of their BCR in order to lose autoreactivity or dysfunctionality[18,19]. Receptor editing can be initiated in immature B cells upon strong signaling of an autoreactive BCR or by impaired tonic signaling of nonfunctional BCRs[17]. This triggering leads to a secondary recombination event of antibody genes encoding the variable domain of the LC. This rearrangement can involve a new VJ recombination event on the original kappa locus with genes positioned distally from the original gene. Alternatively, rearrangement can take place at the second kappa locus or on one of the lambda loci. If receptor editing fails to correct autoreactivity or unproductivity of gene rearrangement, B cells will eventually undergo apoptosis in a process termed clonal deletion[20].

It is important to note that central tolerance is not complete and a fraction of autoreactive B cells generated in the bone marrow reach the circulation[16,21]. Therefore, additional measures in the periphery are required to prevent unwarranted autoreactive B cell responses.

B cell activation

Naïve B cells are continuously circulating through lymph and blood to search for antigen. To initiate a B cell response, B cells have to encounter their cognate antigen[22]. These encounters are predominantly taking place in secondary lymphoid organs, such as lymph nodes, spleen and mucosa-associated lymphoid tissues. These tissues filter body fluids, retain antigen and display the antigen to B cells[23,24].

The activation of B cells is initiated when antigen triggers the BCR. Signaling of the BCR induces multiple cellular effects, including survival, migration and proliferation[25]. Commonly, complex antigens amplify these cellular effects by their ability to trigger additional receptors including toll-like receptors and complement receptor 2[26]. Depending on the nature of the antigen encountered, B cell activation can occur through multiple pathways (Fig. 3). A B cell response can be initiated without the help of T cells if the antigen induces strong activation of the BCR combined with triggering of co-receptors[27]. This T cell-independent pathway is triggered by highly multivalent antigens, such as polysaccharides. More commonly, B cells require the help of T cells to generate a robust B cell response.

The help of T cells is acquired by B cells through the presentation of antigen on MHC class II[28]. B cells present their encountered antigen by internalization of the antigen upon BCR

triggering. Internalized antigen is processed and loaded onto MHC class II molecules to facilitate the interaction with $CD4^+$ T cells. If a rare antigen-specific T cell recognizes the antigen presented by a B cell, the T cell will provide signals that further activate B cells[29,30].

Extrafollicular versus germinal center pathway

The initial stimulation with antigen and subsequent interaction with T cells determine whether B cell differentiation continues through the extrafollicular or follicular pathway[31]. The extrafollicular pathways typically involves the direct differentiation of naïve B cells to memory B cells and short-lived plasmablasts[32]. These plasmablasts secrete antibodies within the first days upon antigen exposure and, thereby, provide a first wave of antibodies.

B cell differentiation can also continue in germinal centers (GCs). GCs are structures containing aggregates of B cells which are typically formed a few days upon antigen encounter[6]. GCs have the unique ability to enhance the affinity of B cells towards their antigen in a highly regulated process termed affinity maturation. This process involves cycling of B cells through two separate zones of the GC: the dark zone and the light zone[33]. In the dark zone, B cells proliferate and undergo somatic hypermutation in which mutations in the genes encoding the variable domains of the antibodies are acquired. Somatic hypermutation diversifies the BCR and changes their relative affinity for the antigen. Subsequently, B cells migrate from the dark zone to the light zone. In the light zone, B cells with the highest affinity for the antigen are selected[34,35]. This selection involves antigen capturing of the B cells followed by the presentation to T cells residing in the light zone of the GC. B cells with the highest affinity acquire more T cell help and can recycle though the germinal center to further refine their BCR[6]. Alternatively, selected B cells can exit the GC and differentiate towards memory B cells or antibody-secreting cells.

The extrafollicular and germinal center pathway differ considerably in terms of dynamics and output[22]. The extrafollicular pathway is transient and typically resolves in days, while germinal center reactions can persist for weeks to months[40]. The antibody-secreting cells generated by the extrafollicular pathway are commonly short-lived, whereas the germinal center also generates long-lived plasma cells that home to the bone marrow and can persist for decades[41]. Memory B cells derived from the extrafollicular pathway only have limited mutations and commonly have a lower affinity for antigen in comparison to memory B cells generated by germinal center reactions which are affinity matured[32].

Isotype switching is another process during B cells differentiation which is mainly associated with the GC pathway, but also occurs in the extrafollicular pathway[36]. Isotype switching changes the constant domain of antibodies expressed by B cells and, thereby, affects BCR signaling and effector functions of antibodies[25,37,38]. Naïve B cells express their BCR as IgM or IgD. Upon activation of B cells and subsequent T cell interaction, B cells can change their isotype by switching the coding region for the constant domain to another downstream exon through DNA recombination[37]. Isotype switching is, as upstream DNA is excised, a one-way process based on the order of the sequences encoding the isotypes. In human, the order of isotypes is: IgM, IgD, IgG3, IgG1, IgA1, IgG2, IgG4, IgE, IgA2. B cells are able to undergo sequential class switching. Class switching is regulated by various

triggers, for examples cytokines derived from T cells, to generate antibodies of a certain isotype which are useful in particular immune responses[39].

Peripheral tolerance

The activation and maturation of B cells in the periphery generates robust and high-affine B cell responses which are warranted for protective responses, but should be prevented for autoreactive B cells. Peripheral tolerance mechanisms are designed to prevent the activation of autoreactive B cells that escape central tolerance mechanisms[42].

An important peripheral tolerance mechanism limiting autoreactive B cells is the deletion of autoreactive B cells who encounter self-antigen in the periphery[43,44]. These mechanisms mainly affect transitional B cells which are more sensitive to undergo apoptosis[13,45]. In addition to deletion, autoreactive B cells can become anergic if they encounter self-antigen and, thereby, lose their ability to respond to stimulatory signals[46,47].

Autoreactive B cells can also be limited in their ability to acquire sufficient stimulatory signals to surpass the threshold required to activate naïve B cells. Low affinity for or limited exposure to self-antigen can prevent the activation of autoreactive B cells[48,49]. In addition, if autoreactive B cells are activated by self-antigen, T cell help might not be readily available due to T cell tolerance mechanisms or by effects of regulatory T cells[50-53]. Moreover, activation of autoreactive B cells can also be restricted through signal from inhibitory receptors, such as PD-1, CD22, CD32, LAIR1 and CD95[54].

Autoreactive B cells can also lose their reactivity towards self-antigen in the periphery in a process termed clonal redemption[55,56]. This process is based on somatic hypermutation by which mutations can be introduced that reduce or abolish the reactivity towards self-antigen and render or improve reactivity towards foreign proteins[57]. Thereby, clonal redemption can turn potentially harmful autoreactive B cells into protective B cells.

Effector functions of B cells

B cells can exert multiple effector functions. Antibodies are the most well-known effector molecules of B cells and are secreted by terminally-differentiated B cells, the antibody-secreting cells (including both plasmablasts and plasma cells). Secreted antibodies can be distinguished based on their isotype[3]. IgM is predominantly secreted as pentamer, but can also be hexameric, and is typically the first detectable antibody upon initiation of a B cell response. IgG is a monomeric antibody and is the most abundant antibody in the circulation. IgA can be secreted as monomer or dimer and is associated with mucosal responses. IgE, a monomer, has an important function in allergic responses. IgD is the least abundant secreted antibody and its biological role is unclear.

Secreted antibodies can exert multiple effector functions (Fig. 4). The binding of antibodies to antigen can by itself affect the biological function of the antigen[58]. Thereby, antibodies can block the interaction of the antigen with other molecules, for example to prevent binding of virus particles to host cells. In addition, upon binding to antigen, antibodies

can recruit other immune components using their Fc region. Thereby, antibodies can activate the complement system to opsonize the antigen, recruit immune cells and lyse a microbe[59]. Antibodies can also activate many immune cells, for example to induce cellular phagocytosis of microbes or immune complexes by (amongst others) macrophages or to induce lysis of target cells by natural killer cells[60,61].

Besides the production of antibodies, B cells can also activate T cells. B cells activate T cells by presentation of antigen on HLA class II molecules and by expressing co-stimulatory molecules, like CD80 and CD86, which are critical for the activation of T cells[28,62]. Interactions between B and T cells can have a plethora of effects on T cells[63]. For example, B cells are important for the initial activation of T cells, homing of T cells to tissues, activation of T cells locally and to sustain tissue-resident T cells[64-67]. In addition, B cells mediate the differentiation of T cells towards T follicular helper cells which fulfill a critical role in germinal center reactions[35].

Cytokine production is another effector mechanism of B cells. B cells can produce a variety of cytokines, including the pro-inflammatory cytokines IL-4, IL-6, IL-12, TNF-α and IFN-γ[68-71]. Certain B cells can also produce anti-inflammatory cytokines, such as IL-10 and TGF-ß[72,73].

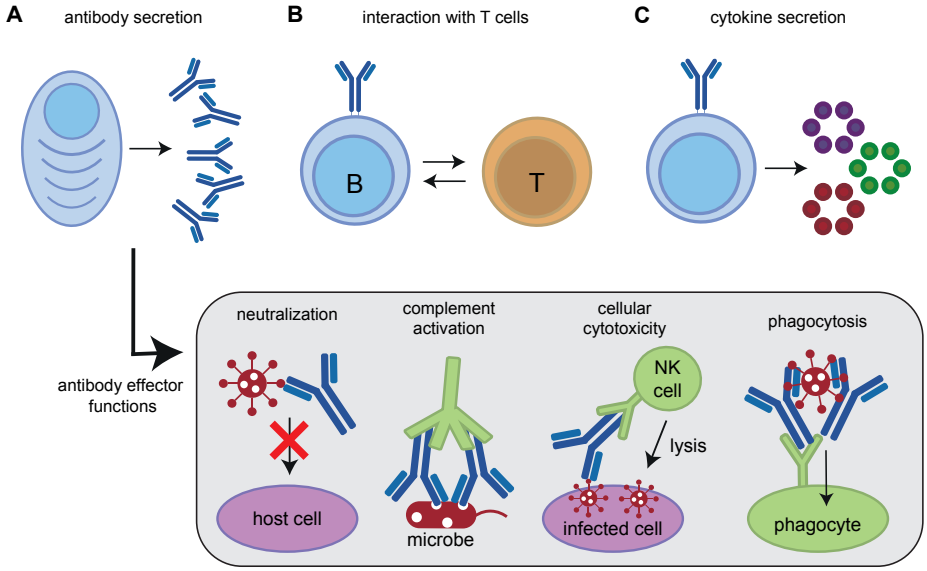

Figure 4. Effector functions of B cells and antibodies. (A) Antibodies are produced by terminally differentiated B cells, the antibody-secreting cells. Antibodies can exert multiple effector function, including microbe neutralization, complement activation and activation of multiple immune cells to lyse infected cells (cellular cytotoxicity) or to take up microbes (phagocytosis). **(B)** B cell can activate T cells by presenting antigen and expressing co-stimulatory molecules. **(C)** B cells can produce a plethora of cytokines which can activate, recruit and regulate other immune cells.

B cell memory and reactivation

B cell responses have the ability to provide lifelong immunity against previously

encountered antigens. Immunological B cell memory is dependent on memory B cells and long-lived plasma cells generated in the primary response[74]. Long-lived plasma cells can persist for decades in specialized niches in lymphoid tissues, particularly the bone marrow[75]. These plasma cells continuously secrete antibodies that can protect against recurrent infections. Memory B cells, on the other hand, are quiescent cells mainly residing in the circulation, bone marrow and other secondary lymphoid organs[76]. Antigen encounter triggers strong and quick reactivation of memory B cells. Differentiation of memory B cells to plasmablasts upon reactivation provides a quick and strong peak of antibodies[77]. Alternatively, reactivated memory B cells can enter germinal center reactions to mount further expansion and maturation of the B cell response[78].

Autoimmunity and autoimmune diseases: Break in B cell tolerance

Processes underlying the generation of B cell responses (as described above) are tailored to provide immunological protection and attempt to prevent the generation of autoreactive B cell responses. Nevertheless, incomplete B cell tolerance mechanisms can lead to the generation of autoreactive B cell responses[42]. The presence of such autoreactive B cell responses, typically accompanied by autoantibodies, are a hallmark of autoimmune diseases[79].

Autoimmune diseases are commonly associated with disease-specific autoreactive B cells response targeting a particular antigen[80]. There are many self-proteins that can be targeted by autoreactive B cell responses. For example, an autoreactive B cell response targeting citrullinated proteins is highly specific for rheumatoid arthritis (RA) and a B cell response targeting the autoantigen topoisomerase 1 (TOP1) is strongly associated with systemic sclerosis (SSc)[81,82].

Role of autoreactive B cells in autoimmune diseases

Autoreactive B cell responses play a role in the pathogenesis of autoimmune diseases. Autoantibodies can often be detected years before disease onset[83-85]. The disease-specific presence of autoreactive B cell responses links these responses directly to the pathogenesis of diseases and allows the use of certain autoantibodies as diagnostic or prognostic biomarkers[80]. Moreover, the presence of particular autoantibodies may also associate with clinical disease subtypes, disease severity or involvement of certain organs[81,82,86]. In SSc, for example, presence of antibodies targeting centromere protein B (CENPB) is associated with gastrointestinal involvement and relative mild disease, whereas the presence of antibodies directed against TOP1 is associated with interstitial lung disease impairing lung function and a poor prognosis[82]. Together, these clinical associations favor the hypothesis that autoreactive B cells play an important role in autoimmune diseases.

Another strong indication for the active involvement of autoreactive B cells in the pathogenesis of autoimmune diseases is the effectiveness of B cell targeting therapies in these diseases. Rituximab, a CD20-targeting monoclonal antibody which depletes circulating B cells almost completely, is an effective treatment in multiple autoimmune diseases, including RA and SSc[87-89]. More recently, B cell depletion by CD19-directed

chimeric antigen receptor (CAR) T cells have shown even more promising therapeutic effects[90-94]. Although these B cell targeting therapies broadly deplete B cells and do not only target autoreactive B cells, they support a potential role for autoreactive B cells in autoimmune diseases.

The relation between the presence and traits of disease-specific autoreactive B cell responses and clinical features varies per disease. Such relations probably depend on the exact role of autoreactive B cells in the pathophysiology of the particular autoimmune disease[95]. In some autoimmune diseases, autoantibodies have a pathogenic role[80]. For example, anti-myeloperoxidase autoantibodies are thought to directly mediate disease in anti-neutrophil cytoplasmic autoantibody-associated vasculitis[96-98]. Alternatively, autoreactive B cells can contribute to autoimmune diseases by activation of T cells and production of pro-inflammatory cytokines[99-104]. Although autoreactive B cells can exert these effector functions, the relative contribution of these effector mechanisms and the exact mechanism of how B cells are involved in the pathogenesis of autoimmune diseases are poorly understood.

Induction of autoreactive B cell responses

The existence of B cell autoreactivity in autoimmune diseases, despite the tolerance mechanisms in play, raises questions on how autoreactive B cell responses are induced. B cell tolerance is often broken before the onset of disease, as autoantibodies are commonly present before disease onset[83-85]. Hypothetically, autoreactive B cells may have a BCR that is self-reactive from origin or have acquired autoreactivity in the periphery through introduction of mutations. While autoreactivity has been described in the naïve repertoire, other autoantibodies lose self-reactivity when reversed to germline configuration[55,105,106]. The acquisition of autoreactivity through mutations might depend on the exposure of B cells to antigens of microbial origin with structural resemblance to a self-antigen[107]. In fact, cross-reactivity of autoreactive BCRs and antibodies towards microbial proteins are described in the context of multiple diseases[105,108,109]. By recognition and presentation of microbial antigen, autoreactive B cells might recruit help from pathogen-directed T cells[110,111]. T cells likely help in the initiation and further development of autoreactive B cell responses, based on the presence of class-switching, acquisition of somatic hypermutations, epitope spreading and HLA associations described for autoreactive B cell responses[81,82,86]. Despite these clues, the exact mechanisms underlying the break in B cell tolerance in autoimmune diseases remain to be elucidated.

Chronicity of autoreactive B cell responses

Autoimmune diseases are chronic diseases and, to date, no curative treatments exist. Current treatment strategies are focused on achieving remission of disease through administration of anti-inflammatory drugs. Therapy-free remission is rare and flares of disease are common. The chronic character of autoimmune diseases indicates the chronic presence of disease drivers[112]. Autoreactive B cell responses are persistent and often exhibit features of chronic activation[102,113,114]. Long-lived antibody-secreting cells could be responsible for the persistence of circulating autoantibodies. In addition, continuous

exposure to self-antigen and stimulatory signals from T cells might continuously trigger autoreactive memory B cells. In line, a reset of the immune system through autologous stem cell transplantation or anti-CD19 CAR T cell therapy is effective[115,116]. This indicates that persistent autoreactive B cell responses might be a chronic driver of autoimmune diseases.

Systemic sclerosis

SSc is a complex autoimmune disease which is clinically characterized by vasculopathy and fibrosis of the skin and internal organs[117]. Although the disease is rare, it has a high morbidity and mortality. The clinical presentation of the disease is highly heterogeneous and the severity and progression of the disease strongly varies between patients. Curative treatments for SSc do not exist and the management of the disease focuses on inhibiting disease progression using various medications, including immunosuppressive drugs, fibrosis inhibitors and autologous stem cell transplantation[118]. Enhanced understanding of the mechanisms underlying the disease are warranted to understand the development of disease complications and to enable effective treatment.

A hallmark of SSc is the presence of autoreactive B cell responses targeting nuclear antigens[82]. The role of autoreactive B cell responses in this disease is reviewed in detail in chapter 2. In short, autoreactive B cells can target a variety of nuclear antigens, such as TOP1 and CENPB. These autoreactive B cell responses, which rarely co-occur in SSc patients, strongly associate with specific disease subtypes and disease complications[82]. Moreover, SSc is responsive to B cell depletion[88,92-94,116]. Together, these findings indicate that autoreactive B cells probably play an important role in SSc.

Rheumatoid arthritis

RA is a chronic autoimmune disease that primarily affects the joints[119]. The disease is common with a prevalence ranging between 0.25% and 1%[120]. Clinically, RA is characterized by inflammation of synovial tissue in the joints which causes joint pain and swelling. The treatment of RA is focused on the inhibition of inflammation by the use of disease modifying-antirheumatic drugs (DMARDs)[121]. Despite these medications, the disease persists and flares occur. Therefore, it is crucial to understand the chronic drivers of RA to improve treatment and eventually cure the disease.

RA is hallmarked by an autoreactive B cell response targeting post-translationally modified proteins, in particular citrullinated, carbamylated and acetylated proteins[81]. These autoantibodies are highly specific for RA and are present in about 50-70% of patients. The B cell response targeting post-translationally modified proteins is closely linked to disease onset and associated with a higher risk for disease relapse and low incidence of drug-free sustained remission[122-124]. In addition, RA is responsive to B cell depletion[87,89,90]. Together, these findings indicate that the B cell response targeting post-translationally modified proteins plays an important role in the pathogenesis of RA.

Thesis outline

Many questions regarding the dysregulation of autoreactive B cells in human autoimmune diseases remain poorly understood. The aim of this thesis is to elucidate mechanisms underpinning the origin, dynamics and role of autoreactive B cell responses in rheumatic autoimmune diseases. Given the importance of B cells in these diseases, insights into these mechanisms underlying their generation, activation and maintenance will enhance the understanding of mechanisms that drive disease. Thereby, these insights could improve clinical management of the disease, identify targets to treat or eventually cure disease, or open possibilities for strategies that could prevent disease.

The first section of this thesis focuses on autoreactive B cells in SSc. As reviewed in **chapter 2**, autoreactive B cells targeting nuclear antigens are likely to play an important role in the pathogenesis of SSc. However, direct assessments of the activity and dynamics of autoreactive B cell responses in SSc are lacking. Therefore, we determined phenotypic and functional characteristics of TOP1-reactive B cells in SSc in **chapter 3**. We found that class-switched TOP1-reactive B cells in SSc patients displayed characteristics of a strongly activated response. These findings point towards active triggering of TOP1-reactive B cells in SSc.

To identify antigenic triggers responsible for the activation of TOP1-reactive B cells, we investigated molecular and functional characteristics of anti-TOP1 autoantibodies (ATAs). In **chapter 4**, we observed a remarkable heterogeneity between ATAs of SSc patients in their binding to TOP1 and TOP1-DNA cleavage complexes (TOP1cc). Enhanced recognition of TOP1cc associated with anti-TOP1-autoantibody titers and interstitial lung disease in SSc patients and potentiated the immunostimulatory properties of particular ATAs. Besides effects of DNA binding, we also investigated the possible role of microbes in triggering TOP1-reactive B cells in SSc in **chapter 5**. We found that a subset of human ATAs can cross-react to a fungal TOP1. This fungal TOP1 could trigger the activation of a TOP1-reactive B cell line. Moreover, presence of circulating fungal TOP1-reactive antibodies associated with disease severity in SSc. Together, these studies indicate that DNA binding modulates the activation of TOP1-reactive B cells in SSc. Moreover, they suggest that human and microbial-derived antigens might trigger TOP1-reactive B cells in SSc.

To explore the relation between the dynamics of an autoreactive B cell response and clinical disease, we studied B cells expressing anti-citrullinated protein antibodies (ACPA) in RA in **chapter 6**. ACPA-expressing B cells displayed a highly activated phenotype irrespective of clinical disease control in patients treated with different anti-inflammatory drugs. This persistent immunological activity might explain the chronic character of RA. In **chapter 7**, a method to identify ACPA-expressing B cells with low surface BCR expression was developed. This method can be used to track the dynamics of autoreactive B cells in more detail.

Finally, the findings described in this thesis are summarized in **chapter 8**, which highlights the relevance of these insights for understanding the biology of autoreactive B cell responses in rheumatic autoimmune diseases.

References:

1 Briney, B., Inderbitzin, A., Joyce, C. & Burton, D. R. Commonality despite exceptional diversity in the baseline human antibody repertoire. *Nature* **566**, 393–397 (2019).
2 Soto, C. *et al*. High frequency of shared clonotypes in human B cell receptor repertoires. *Nature* **566**, 398–402 (2019).
3 Schroeder, H. W., Jr. & Cavacini, L. Structure and function of immunoglobulins. *J Allergy Clin Immunol* **125**, S41–52 (2010).
4 Schatz, D. G. & Ji, Y. Recombination centres and the orchestration of V(D)J recombination. *Nat Rev Immunol* **11**, 251–263 (2011).
5 Bassing, C. H., Swat, W. & Alt, F. W. The mechanism and regulation of chromosomal V(D)J recombination. *Cell* **109**, S45–55 (2002).
6 Victora, G. D. & Nussenzweig, M. C. Germinal centers. *Annu Rev Immunol* **40**, 413–442 (2022).
7 Kondo, M., Weissman, I. L. & Akashi, K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* **91**, 661–672 (1997).
8 Miller, J. P. *et al*. The earliest step in B lineage differentiation from common lymphoid progenitors is critically dependent upon interleukin 7. *J Exp Med* **196**, 705–711 (2002).
9 Maki, R., Kearney, J., Paige, C. & Tonegawa, S. Immunoglobulin gene rearrangement in immature B cells. *Science* **209**, 1366–1369 (1980).
10 Wang, Y. H. *et al*. Differential surrogate light chain expression governs B-cell differentiation. *Blood* **99**, 2459–2467 (2002).
11 Ehlich, A. *et al*. Immunoglobulin heavy and light chain genes rearrange independently at early stages of B cell development. *Cell* **72**, 695–704 (1993).
12 Lam, K. P., Kuhn, R. & Rajewsky, K. *In vivo* ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. *Cell* **90**, 1073–1083 (1997).
13 Levine, M. H. *et al*. A B-cell receptor-specific selection step governs immature to mature B cell differentiation. *Proc Natl Acad Sci U S A* **97**, 2743–2748 (2000).
14 Allman, D. & Pillai, S. Peripheral B cell subsets. *Curr Opin Immunol* **20**, 149–157 (2008).
15 Meffre, E. *et al*. Surrogate light chain expressing human peripheral B cells produce self-reactive antibodies. *J Exp Med* **199**, 145–150 (2004).
16 Wardemann, H. *et al*. Predominant autoantibody production by early human B cell precursors. *Science* **301**, 1374–1377 (2003).
17 Nemazee, D. Mechanisms of central tolerance for B cells. *Nat Rev Immunol* **17**, 281–294 (2017).
18 Okoreeh, M. K. *et al*. Asymmetrical forward and reverse developmental trajectories determine molecular programs of B cell antigen receptor editing. *Sci Immunol* **7**, eabm1664 (2022).
19 Retter, M. W. & Nemazee, D. Receptor editing occurs frequently during normal B cell development. *J Exp Med* **188**, 1231–1238 (1998).
20 Hartley, S. B. *et al*. Elimination of self-reactive B lymphocytes proceeds in two stages: arrested development and cell death. *Cell* **72**, 325–335 (1993).
21 Yurasov, S. *et al*. Defective B cell tolerance checkpoints in systemic lupus erythematosus. *J Exp Med* **201**, 703–711 (2005).
22 Cyster, J. G. & Allen, C. D. C. B cell responses: cell interaction dynamics and decisions. *Cell* **177**, 524–540 (2019).
23 Cyster, J. G. B cell follicles and antigen encounters of the third kind. *Nat Immunol* **11**, 989–996 (2010).
24 Heesters, B. A., Myers, R. C. & Carroll, M. C. Follicular dendritic cells: dynamic antigen libraries. *Nat Rev Immunol* **14**, 495–504 (2014).
25 Degn, S. E. & Tolar, P. Towards a unifying model for B-cell receptor triggering. *Nat Rev Immunol* **25**, 77–91 (2025).
26 Browne, E. P. Regulation of B-cell responses by Toll-like receptors. *Immunology* **136**, 370–379 (2012).
27 Mond, J. J., Lees, A. & Snapper, C. M. T cell-independent antigens type 2. *Annu Rev Immunol* **13**,

655–692 (1995).
28 Batista, F. D. & Harwood, N. E. The who, how and where of antigen presentation to B cells. *Nat Rev Immunol* **9**, 15–27 (2009).
29 Crotty, S. A brief history of T cell help to B cells. *Nat Rev Immunol* **15**, 185–189 (2015).
30 Lee, S. K. *et al*. B cell priming for extrafollicular antibody responses requires Bcl-6 expression by T cells. *J Exp Med* **208**, 1377–1388 (2011).
31 Faliti, C. E. *et al*. Interleukin-2-secreting T helper cells promote extra-follicular B cell maturation via intrinsic regulation of a B cell mTOR-AKT-Blimp-1 axis. *Immunity* **57**, 2772–2789 e2778 (2024).
32 Eisenbarth, S. C. *et al*. A roadmap for defining "extrafollicular" B cell responses. *Immunity* **58**, 2627-2645 *(*2025).
33 Victora, G. D. *et al*. Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. *Cell* **143**, 592–605 (2010).
34 Chen, S. T., Oliveira, T. Y., Gazumyan, A., Cipolla, M. & Nussenzweig, M. C. B cell receptor signaling in germinal centers prolongs survival and primes B cells for selection. *Immunity* **56**, 547–561 e547 (2023).
35 Crotty, S. T Follicular Helper Cell Biology: A Decade of Discovery and Diseases. *Immunity* **50**, 1132–1148 (2019).
36 Roco, J. A. *et al*. Class-switch recombination occurs infrequently in germinal centers. *Immunity* **51**, 337–350 e337 (2019).
37 Chaudhuri, J. & Alt, F. W. Class-switch recombination: interplay of transcription, DNA deamination and DNA repair. *Nat Rev Immunol* **4**, 541–552 (2004).
38 Vidarsson, G., Dekkers, G. & Rispens, T. IgG subclasses and allotypes: from structure to effector functions. *Front Immunol* **5**, 520 (2014).
39 Schultz, C. L. & Coffman, R. L. Control of isotype switching by T cells and cytokines. *Curr Opin Immunol* **3**, 350–354 (1991).
40 Kim, W. *et al*. Germinal centre-driven maturation of B cell response to mRNA vaccination. *Nature* **604**, 141–145 (2022).
41 Liu, X., Yao, J., Zhao, Y., Wang, J. & Qi, H. Heterogeneous plasma cells and long-lived subsets in response to immunization, autoantigen and microbiota. *Nat Immunol* **23**, 1564–1576 (2022).
42 Reijm, S., Kissel, T. & Toes, R. E. M. Checkpoints controlling the induction of B cell mediated autoimmunity in human autoimmune diseases. *Eur J Immunol* **50**, 1885–1894 (2020).
43 Andrews, S. F. *et al*. Global analysis of B cell selection using an immunoglobulin light chain-mediated model of autoreactivity. *J Exp Med* **210**, 125–142 (2013).
44 Russell, D. M. *et al*. Peripheral deletion of self-reactive B cells. *Nature* **354**, 308–311 (1991).
45 Carsetti, R., Kohler, G. & Lamers, M. C. Transitional B cells are the target of negative selection in the B cell compartment. *J Exp Med* **181**, 2129–2140 (1995).
46 Goodnow, C. C. *et al*. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. *Nature* **334**, 676–682 (1988).
47 Merrell, K. T. *et al*. Identification of anergic B cells within a wild-type repertoire. *Immunity* **25**, 953–962 (2006).
48 Adelstein, S. *et al*. Induction of self-tolerance in T cells but not B cells of transgenic mice expressing little self antigen. *Science* **251**, 1223–1225 (1991).
49 Huang, H., Kearney, J. F., Grusby, M. J., Benoist, C. & Mathis, D. Induction of tolerance in arthritogenic B cells with receptors of differing affinity for self-antigen. *PNAS* **103**, 3734–3739 (2006).
50 Akkaya, M. *et al*. Second signals rescue B cells from activation-induced mitochondrial dysfunction and death. *Nat Immunol* **19**, 871–884 (2018).
51 Akama-Garren, E. H. *et al*. T cell help shapes B cell tolerance. *Sci Immunol* **9**, eadj7029 (2024).
52 Dikiy, S. & Rudensky, A. Y. Principles of regulatory T cell function. *Immunity* **56**, 240–255 (2023).
53 Linterman, M. A. *et al*. Foxp3+ follicular regulatory T cells control the germinal center response.

Nat Med **17**, 975–982 (2011).
54 Getahun, A. Role of inhibitory signaling in peripheral B cell tolerance. *Immunol Rev* **307**, 27–42 (2022).
55 Reed, J. H., Jackson, J., Christ, D. & Goodnow, C. C. Clonal redemption of autoantibodies by somatic hypermutation away from self-reactivity during human immunization. *J Exp Med* **213**, 1255–1265 (2016).
56 Sabouri, Z. *et al*. Redemption of autoantibodies on anergic B cells by variable-region glycosylation and mutation away from self-reactivity. *Proc Natl Acad Sci U S A* **111**, E2567–2575 (2014).
57 Burnett, D. L. *et al*. Germinal center antibody mutation trajectories are determined by rapid self/foreign discrimination. *Science* **360**, 223–226 (2018).
58 Burton, D. R. Antiviral neutralizing antibodies: from *in vitro* to *in vivo* activity. *Nat Rev Immunol* **23**, 720–734 (2023).
59 Goldberg, B. S. & Ackerman, M. E. Antibody-mediated complement activation in pathology and protection. *Immunol Cell Biol* **98**, 305–317 (2020).
60 Zahavi, D., AlDeghaither, D., O'Connell, A. & Weiner, L. M. Enhancing antibody-dependent cell-mediated cytotoxicity: a strategy for improving antibody-based immunotherapy. *Antib Ther* **1**, 7–12 (2018).
61 Tay, M. Z., Wiehe, K. & Pollara, J. Antibody-Dependent Cellular Phagocytosis in Antiviral Immune Responses. *Front Immunol* **10**, 332 (2019).
62 Hathcock, K. S., Laszlo, G., Pucillo, C., Linsley, P. & Hodes, R. J. Comparative analysis of B7-1 and B7-2 costimulatory ligands: expression and function. *J Exp Med* **180**, 631–640 (1994).
63 Ghosh, D., Jiang, W., Mukhopadhyay, D. & Mellins, E. D. New insights into B cells as antigen presenting cells. *Curr Opin Immunol* **70**, 129–137 (2021).
64 Hong, S. *et al*. B cells are the dominant antigen-presenting cells that activate naive CD4(+) T cells upon immunization with a virus-derived nanoparticle antigen. *Immunity* **49**, 695–708 e694 (2018).
65 Naderi, W., Schreiner, D. & King, C. G. T-cell-B-cell collaboration in the lung. *Curr Opin Immunol* **81**, 102284 (2023).
66 Ritzau-Jost, J. & Hutloff, A. T cell/B cell interactions in the establishment of protective immunity. *Vaccines (Basel)* **9**, 1074 (2021).
67 Laderach, F. *et al*. EBV induces CNS homing of B cells attracting inflammatory T cells. *Nature* **646**, 171-179 (2025).
68 Arkatkar, T. *et al*. B cell-derived IL-6 initiates spontaneous germinal center formation during systemic autoimmunity. *J Exp Med* **214**, 3207–3217 (2017).
69 Duddy, M. E., Alter, A. & Bar-Or, A. Distinct profiles of human B cell effector cytokines: a role in immune regulation? J Immunol **172**, 3422–3427 (2004).
70 Harris, D. P. *et al*. Reciprocal regulation of polarized cytokine production by effector B and T cells. *Nat Immunol* **1**, 475–482 (2000).
71 Lund, F. E. Cytokine-producing B lymphocytes-key regulators of immunity. *Curr Opin Immunol* **20**, 332–338 (2008).
72 Fillatreau, S., Sweenie, C. H., McGeachy, M. J., Gray, D. & Anderton, S. M. B cells regulate autoimmunity by provision of IL-10. *Nat Immunol* **3**, 944–950 (2002).
73 Lee, K. M. *et al*. TGF-beta-producing regulatory B cells induce regulatory T cells and promote transplantation tolerance. *Eur J Immunol* **44**, 1728–1736 (2014).
74 Akkaya, M., Kwak, K. & Pierce, S. K. B cell memory: building two walls of protection against pathogens. *Nat Rev Immunol* **20**, 229–238 (2020).
75 Khodadadi, L., Cheng, Q., Radbruch, A. & Hiepe, F. The maintenance of memory plasma cells. *Front Immunol* **10**, 721 (2019).
76 Inoue, T. & Kurosaki, T. Memory B cells. *Nat Rev Immunol* **24**, 5–17 (2024).
77 Syeda, M. Z., Hong, T., Huang, C., Huang, W. & Mu, Q. B cell memory: from generation to

reactivation: a multipronged defense wall against pathogens. *Cell Death Discov* **10**, 117 (2024).
78 Turner, J. S. *et al*. Human germinal centres engage memory and naive B cells after influenza vaccination. *Nature* **586**, 127–132 (2020).
79 Rubin, S. J. S., Bloom, M. S. & Robinson, W. H. B cell checkpoints in autoimmune rheumatic diseases. *Nat Rev Rheumatol* **15**, 303–315 (2019).
80 Volkov, M. *et al*. Comprehensive overview of autoantibody isotype and subclass distribution. *J Allergy Clin Immunol* **150**, 999–1010 (2022).
81 Scherer, H. U., van der Woude, D. & Toes, R. E. M. From risk to chronicity: evolution of autoreactive B cell and antibody responses in rheumatoid arthritis. *Nat Rev Rheumatol* **18**, 371–383 (2022).
82 Liem, S. I. E. *et al*. Autoreactive B cell responses targeting nuclear antigens in systemic sclerosis: Implications for disease pathogenesis. *Semin Arthritis Rheum* **58**, 152136 (2023).
83 Bellando-Randone, S. *et al*. Progression of patients with Raynaud's phenomenon to systemic sclerosis: a five-year analysis of the European Scleroderma Trial and Research group multicentre, longitudinal registry study for Very Early Diagnosis of Systemic Sclerosis (VEDOSS). Lancet Rheumatol **3**, e834–e843 (2021).
84 Rantapaa-Dahlqvist, S. *et al*. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* **48**, 2741–2749 (2003).
85 Arbuckle, M. R. *et al*. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med* **349**, 1526–1533 (2003).
86 Pisetsky, D. S. & Lipsky, P. E. New insights into the role of antinuclear antibodies in systemic lupus erythematosus. *Nat Rev Rheumatol* **16**, 565–579 (2020).
87 Chatzidionysiou, K. *et al*. Highest clinical effectiveness of rituximab in autoantibody-positive patients with rheumatoid arthritis and in those for whom no more than one previous TNF antagonist has failed: pooled data from 10 European registries. *Ann Rheum Dis* **70**, 1575–1580 (2011).
88 Ebata, S. *et al*. Safety and efficacy of rituximab in systemic sclerosis (DESIRES): open-label extension of a double-blind, investigators-initiated, randomised, placebo-controlled trial. *Lancet Rheumatol* **4**, e546–e555 (2022).
89 Emery, P. *et al*. Efficacy and safety of different doses and retreatment of rituximab: a randomised, placebo-controlled trial in patients who are biological naive with active rheumatoid arthritis and an inadequate response to methotrexate (Study Evaluating Rituximab's Efficacy in MTX iNadequate rEsponders (SERENE)). Ann Rheum Dis **69**, 1629–1635 (2010).
90 Lidar, M. *et al*. CD-19 CAR-T cells for polyrefractory rheumatoid arthritis. *Ann Rheum Dis* **84**, 370–372 (2025).
91 Mackensen, A. *et al*. Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus. *Nat Med* **28**, 2124–2132 (2022).
92 Auth, J. *et al*. CD19-targeting CAR T-cell therapy in patients with diffuse systemic sclerosis: a case series. *Lancet Rheumatol* **7**, e83–e93 (2025).
93 Pecher, A. C. *et al*. CD19-targeting CAR T cell therapy in five patients with systemic sclerosis unsuitable for autologous stem cell transplantation. *EULAR Rheumatology Open* **000**, 100008 (2025).
94 Wang, X. *et al*. Allogeneic CD19-targeted CAR-T therapy in patients with severe myositis and systemic sclerosis. *Cell* **187**, 4890–4904 e4899 (2024).
95 Lee, D. S. W., Rojas, O. L. & Gommerman, J. L. B cell depletion therapies in autoimmune disease: advances and mechanistic insights. *Nat Rev Drug Discov* **20**, 179–199 (2021).
96 Falk, R. J., Terrell, R. S., Charles, L. A. & Jennette, J. C. Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals *in vitro*. *PNAS* **87**, 4115–4119 (1990).
97 Kessenbrock, K. *et al*. Netting neutrophils in autoimmune small-vessel vasculitis. *Nat Med* **15**, 623–625 (2009).
98 Xiao, H. *et al*. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause

glomerulonephritis and vasculitis in mice. *J Clin Invest* **110**, 955–963 (2002).
99 Weber, M. S. *et al*. B-cell activation influences T-cell polarization and outcome of anti-CD20 B-cell depletion in central nervous system autoimmunity. *Ann Neurol* **68**, 369–383 (2010).
100 Molnarfi, N. *et al*. MHC class II-dependent B cell APC function is required for induction of CNS autoimmunity independent of myelin-specific antibodies. *J Exp Med* **210**, 2921–2937 (2013).
101 Jelcic, I. *et al*. Memory B cells activate brain-homing, autoreactive CD4(+) T cells in multiple sclerosis. *Cell* **175**, 85–100 e123 (2018).
102 Kristyanto, H. *et al*. Persistently activated, proliferative memory autoreactive B cells promote inflammation in rheumatoid arthritis. *Sci Transl Med* **12**, eaaz5327 (2020).
103 Barr, T. A. *et al*. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. *J Exp Med* **209**, 1001–1010 (2012).
104 Li, R. *et al*. Proinflammatory GM-CSF-producing B cells in multiple sclerosis and B cell depletion therapy. *Sci Transl Med* **7**, 310ra166 (2015).
105 Li, S. *et al*. Autoantibodies From Single Circulating Plasmablasts React With Citrullinated Antigens and *Porphyromonas gingivalis* in Rheumatoid Arthritis. *Arthritis Rheumatol* **68**, 614–626 (2016).
106 Mietzner, B. *et al*. Autoreactive IgG memory antibodies in patients with systemic lupus erythematosus arise from nonreactive and polyreactive precursors. *PNAS* **105**, 9727–9732 (2008).
107 Rojas, M. *et al*. Molecular mimicry and autoimmunity. *J Autoimmun* **95**, 100–123 (2018).
108 Lanz, T. V. *et al*. Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM. Nature **603**, 321–327 (2022).
109 Jacobs, B. C., Hazenberg, M. P., van Doorn, P. A., Endtz, H. P. & van der Meche, F. G. Cross-reactive antibodies against gangliosides and *Campylobacter jejuni* lipopolysaccharides in patients with Guillain-Barre or Miller Fisher syndrome. *J Infect Dis* **175**, 729–733 (1997).
110 Greiling, T. M. *et al*. Commensal orthologs of the human autoantigen Ro60 as triggers of autoimmunity in lupus. *Sci Transl Med* **10**, eaan2306 (2018).
111 Gronke, K. *et al*. Translocating gut pathobiont *Enterococcus gallinarum* induces T(H)17 and IgG3 anti-RNA-directed autoimmunity in mouse and human. *Sci Transl Med* **17**, eadj6294 (2025).
112 Schett, G., Tanaka, Y. & Isaacs, J. D. Why remission is not enough: underlying disease mechanisms in RA that prevent cure. *Nat Rev Rheumatol* **17**, 135–144 (2021).
113 Suurmond, J. *et al*. Patterns of ANA+ B cells for SLE patient stratification. *JCI Insight* **4**, e127885 (2019).
114 Maschmeyer, P. *et al*. Immunological memory in rheumatic inflammation - a roadblock to tolerance induction. *Nat Rev Rheumatol* **17**, 291–305 (2021).
115 Alexander, T. & Greco, R. Hematopoietic stem cell transplantation and cellular therapies for autoimmune diseases: overview and future considerations from the Autoimmune Diseases Working Party (ADWP) of the European Society for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplant* **57**, 1055–1062 (2022).
116 Schett, G. & June, C. H. CAR T cells in autoimmune disease: On the road to remission. *Immunity* **57**, 2705–2709 (2024).
117 Denton, C. P. & Khanna, D. Systemic sclerosis. *Lancet* **390**, 1685–1699 (2017).
118 Pope, J. E. *et al*. State-of-the-art evidence in the treatment of systemic sclerosis. *Nat Rev Rheumatol* **19**, 212–226 (2023).
119 Di Matteo, A., Bathon, J. M. & Emery, P. Rheumatoid arthritis. *Lancet* **402**, 2019–2033 (2023).
120 Finckh, A. *et al*. Global epidemiology of rheumatoid arthritis. *Nat Rev Rheumatol* **18**, 591–602 (2022).
121 Konzett, V. & Aletaha, D. Management strategies in rheumatoid arthritis. *Nat Rev Rheumatol* **20**, 760–769 (2024).
122 van de Stadt, L. A. *et al*. Development of the anti-citrullinated protein antibody repertoire

prior to the onset of rheumatoid arthritis. *Arthritis Rheum* **63**, 3226–3233 (2011).

123 Rech, J. *et al*. Prediction of disease relapses by multibiomarker disease activity and autoantibody status in patients with rheumatoid arthritis on tapering DMARD treatment. *Ann Rheum Dis* **75**, 1637–1644 (2016).

124 Ajeganova, S. *et al*. Disease-modifying antirheumatic drug-free sustained remission in rheumatoid arthritis: an increasingly achievable outcome with subsidence of disease symptoms. *Ann Rheum Dis* **75**, 867–873 (2016).