



Universiteit
Leiden
The Netherlands

Checkpoints controlling the induction of B cell mediated autoimmunity in human autoimmune diseases

Reijm, S.; Kissel, T.; Toes, R.E.M.

Citation

Reijm, S., Kissel, T., & Toes, R. E. M. (2020). Checkpoints controlling the induction of B cell mediated autoimmunity in human autoimmune diseases. *European Journal Of Immunology*, 50(12), 1885-1894. doi:10.1002/eji.202048820

Version: Publisher's Version

License: [Creative Commons CC BY 4.0 license](https://creativecommons.org/licenses/by/4.0/)

Downloaded from: <https://hdl.handle.net/1887/3182406>

Note: To cite this publication please use the final published version (if applicable).

REVIEW**Checkpoints controlling the induction of B cell mediated autoimmunity in human autoimmune diseases**S. Reijm* , T. Kissel*  and R.E.M. Toes* 

Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands

B cell targeting therapies are effective in various autoimmune diseases, among others rheumatoid arthritis, pemphigus vulgaris, and systemic lupus erythematosus. Given these successes, it is evident that B cells are central orchestrators in the processes leading to the signs and symptoms hallmarking many human autoimmune diseases. The pathways provoking the generation of such autoreactive B cells or mechanisms preventing their induction in health are, however, poorly explored. Nevertheless, such information is crucial for the development of preventative/curative interventions aiming to permanently deplete- or prohibit the emergence of autoreactive B cells. Hence, this review will focus on how B cell tolerance might be breached, and which checkpoints are at play preventing the arousal of autoreactive B cells in human. Especially antigen presentation by follicular dendritic cells, somatic hypermutation, and cross-reactivity to the microbiome/environment could operate as actors playing pivotal roles in the induction of B cell-mediated humoral autoimmunity. Moreover, we highlight the human autoimmune disease rheumatoid arthritis as a prototype where autoreactive B cells combine several mechanisms to overcome peripheral B cell checkpoints.

Keywords: autoimmunity · cross presentation/priming · regulatory T cells · rheumatology · tolerance

Introduction

Clear evidence for a key role of B cells in human autoimmune diseases, such as rheumatoid arthritis (RA), pemphigus vulgaris, systemic lupus erythematosus (SLE), myasthenia graves, or anti-neutrophil cytoplasmic antibody-associated vasculitis (AAV), is provided by the efficacy of B cell-targeted therapies in these diseases [1–4]. B cells might contribute in several ways to these disorders, including the production of pathogenic autoantibodies, the generation of inflammatory cytokines, and the presentation of antigens to T cells combined with their excellent ability to activate these cells. The different B cell functions in human autoimmune diseases have been extensively reviewed and will not be discussed in this review [5,6].

During B cell development in the BM, B cells start to express B cell receptors (BCRs) with diverse antigen-binding sites, created through a random combination of variable (V), joining (J), and diversity (D) gene segments [7]. This junctional diversity during V(D)J-recombination is further increased by the introduction of palindromic- and non-templated nucleotides [8, 9]. Additionally, the association between heavy- and light chain creates further diversity in the BCR V-domains. Collectively these processes generate highly diverse BCR repertoires and B cell populations able to recognize many different structures and molecules in a highly specific manner, including self-molecules expressed by healthy human tissues. It is estimated that approximately 50–75% of immature BM B cells carry self-reactive BCRs in both mice and humans [10–12]. A significant proportion of these B cells will undergo receptor-editing or clonal deletion both

Correspondence: Dr. R.E.M. Toes
e-mail: R.E.M.Toes@lumc.nl

*All authors contributed equally.

leading to the removal of self-reactivity as experimentally evidenced within Ig transgenic mouse models [13]. During receptor-editing, autoreactive B cells against multivalent antigens will change their light chain to prevent autoreactivity. If this mechanism fails or if the B cell shows a high affinity to self-antigens, it will most likely undergo clonal deletion. These negative selection processes are responsible for central B cell tolerance and ensure that most B cells leaving the BM do not recognize self. However, as central B cell tolerance is incomplete, self-reactive B cells will escape into the periphery. To further hinder the emergence of autoreactive B cell responses, several complementary peripheral tolerance mechanisms and B cell activation checkpoints are in place to prevent the induction of humoral autoimmunity. Immature self-reactive B cells coming from the BM are, for example, competing with other B cells to enter primary follicles and exclusion will result in cell death within 1–3 days. This follicular exclusion of (auto)antigen-reactive B cells, shown in different mouse systems, will depend on the diversity and abundance of competing cells and is not absolute. Nonetheless, it further minimizes the chance that autoreactive B cells enter the follicle and survive in the periphery [14,15]. After passaging the primary follicles, B cells can enter germinal centers (GC) where they interact with antigens presented by follicular dendritic cells (FDCs) and receive survival signals [16]. The activated B cells are migrating to the T/B cell border to present antigen and receive “help” from T helper cells. These B cells will then undergo proliferation and somatic hypermutation (SHM) in the dark zone following antigen-driven selection by FDCs in the light zone [17,18]. Murine studies have also shown that selected B cells can re-enter or exit the GC as memory B cells or plasmablasts/cells secreting high affinity isotype-switched antibodies. Thus, retained antigen presentation by FDCs and the recruitment of “help” from T helper cells are both essential peripheral checkpoints that are in place to permit the induction of B cell responses by transmitting necessary survival and activation signals to B cells. We will further elaborate on the important role of T cells, antigen presentation, and FDCs as peripheral checkpoints in the main part of the review. Additionally, B cells can also be activated in a T-cell independent manner by bacterial molecules such as LPS. LPS, like other T-cell independent antigens, contain repetitive antigenic structures leading to an extensive BCR crosslinking and also provides additional co-stimulatory signals through Toll-like receptor (TLR) activation. Although the activation of B cells by T-cell independent antigens can induce abundant IgM responses as evidenced by hyper-IgM syndrome, a primary human immune deficiency disorder characterized by defective CD40 signaling, chronic BCR crosslinking with high antigen density in the absence of T cell help or co-stimulation, typically results in B cell anergy. This silencing of B cell clones has been shown to represent an additional peripheral checkpoint in mice as most self-molecules do not provide co-stimulation to B cells [19].

Despite all these checkpoints cooperating in controlling self-reactive B cells (Fig. 1A), negative selection through central and peripheral checkpoints often fails as evidenced by the prevalence of auto-immune diseases in humans that are frequently

characterized by the presence of disease-specific autoantibodies. How or when autoreactive B cells are induced in these diseases remains, however, often obscure. In this review, we will highlight several mechanisms how B cells might bypass peripheral “tolerance” checkpoints including the important role of antigen presentation by FDCs, SHM, and T/B cell cross-reactivity between self and microbial/environmental antigens (Fig. 1B). A special focus will be given to RA, highlighting different mechanisms used by the RA-specific autoreactive B cells producing anti-citrullinated protein antibodies (ACPA) to overrun several B cell checkpoints.

Antigen-presentation and co-stimulation as checkpoint to control autoreactive B cell activation

As noted above, many B cell responses typically undergo an antigen-driven selection controlled by FDCs and follicular T helper cells in the GC. FDCs are essential for efficient GC formation and the generation of high-affinity antibodies as they are a major source of CXCL-13 involved in attracting B and specific T cells to the follicles [20]. Likewise, FDCs also produce factors promoting B cell survival by the supply of IL-6 and the B cell activating factor BAFF [21]. FDCs are stromal derived cells characterized by a dendritic morphology and the expression of various markers such as CD21 (CR2), CD35 (CR1), MFGE8, and Fc γ -receptors [22]. Furthermore, FDCs express multiple TLRs enabling them to respond to viral- or bacterial products in their vicinity. TLR-2 and 4 stimulation of FDCs in the gut induces, for example, the production of TGF- β and BAFF involved in IgA class switching of B cells [23]. Importantly, the expression of high levels of CR1 and CR2 are essential for FDC-mediated antigen retention in a complement dependent manner [24]. FDCs retain internalized antigens, complement coated immune complexes (ICs), within cycling non-degradative endosomal compartments and periodically present them on the cell surface to B cells via CR2 during affinity maturation [22]. In this way, microbes and other particles or molecules that have activated the complement system become preferentially presented to B cells by FDCs. Moreover, such complement-opsonized antigens can also deliver co-stimulatory signals to responding B cells via CD21/CD35 in conjunction with BCR-activation. If this complement dependent co-stimulation is lacking, B cells will not be activated properly and undergo anergy induction [25]. Because FDCs are crucially involved in the regulation of humoral immune responses, they are also an important checkpoint controlling autoreactive B cell responses. In general, most self-antigens will not activate the complement system and hence will not be presented efficiently by FDCs and/or recognized by B cells in a way allowing concurrent co-stimulation and BCR-engagement. Indeed, antigens conditionally expressed by FDCs in a Fc- and complement receptor independent manner, induce B cell tolerance in a mouse model, indicating that FDC can play an important role in B cell tolerance induction to sequestered self-antigens [26]. Nonetheless, some self-constituents, such as apoptotic cells or DNA-complexes, can induce complement activation under certain pathological

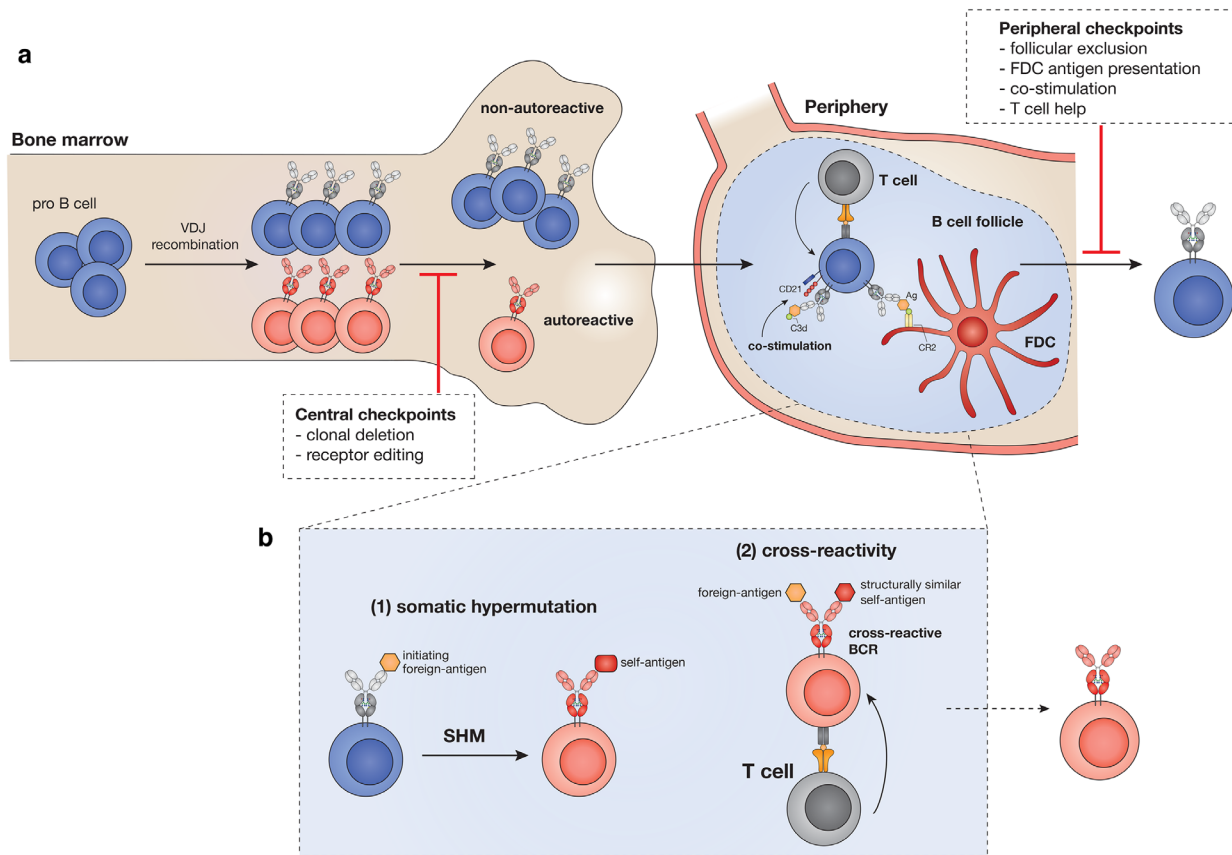


Figure 1. B cell checkpoints and mechanisms to escape. (a) Schematic representation of the B cell development in the BM and the periphery including the respective checkpoints. Random V(D)J recombination of the B cell receptor in the BM can induce autoreactive B cells. Clonal deletion and receptor editing as main central checkpoints eliminate most of these autoreactive B cell clones. B cells that emerge into the periphery have to overcome additional checkpoints including B cell follicular exclusion, antigen presentation by follicular dendritic cells (FDCs), co-stimulation by, e.g., complement-C3d coated antigens, and T cell help. (b) Proposed main mechanisms of B cells to overrun peripheral checkpoints. The generation of self-reactive B cells via SHM is one possible mechanism. B cells will alter their receptor reactivity towards self-antigens. Secondly, cross-reactive BCRs, able to recognize foreign and structurally similar self-antigens, can overrun important gatekeepers as they receive “help” from non-autoreactive T cells.

conditions, potentially leading to the retainment of these antigens by FDCs and induction of B cell autoimmunity [27,28]. The latter could be strengthened further through the ability of FDCs to attract follicular T helper cells, required for the efficient formation of T-B cell interactions and productive GC reactions [29]. Indeed, the important role of FDCs is also suggested by the frequent presence of ectopic lymphoid, GC-like, structures in chronically inflamed tissues of several human autoimmune diseases such as the inflamed synovium of patients with RA [30, 31]. Collectively, these findings identify FDCs as important gatekeepers governing the induction of (unwanted) B cell responses through the control of antigen-presentation and provision of activation- and survival signals.

De novo autoreactivity gained through somatic hypermutation

When a mature B cell recognizes its antigen, it becomes activated and can undergo SHM. During this process, random mutations

might result in an enhanced antigen recognition, leading to avidity maturation of the antibody response through the selection of B cells with the highest antigen affinity. This random mutation process also bears the risk that it might lead to a shift in antigen recognition towards other (structurally similar) self-antigens. However, many such B cells will undergo apoptosis as they compete with other responding B cells for survival signals provided by FDCs and follicular T helper cells. As a consequence, many B cells will mutate away from self-reactivity as it provides a means to overcome energy [32].

Nonetheless, the development of autoimmunity in humans as a consequence of SHM has been described in several studies. For example, in pemphigus vulgaris (PV), an autoimmune disease characterized by blistering and autoantibodies directed against desmoglein (DSG) proteins 1 and 3 [33–35], DSG3-specific antibodies were reverted to their respective germline sequence. As no reactivity to DSG3 could be detected anymore when these antibodies were expressed in germline configuration, the data suggest that the DSG3 reactive B cell was initially activated by another, DSG3-unrelated antigen. Interestingly, one monoclonal antibody

(mAb) expressed only 4 aa replacements, implying that no more than a few mutations are needed to gain autoreactivity [36]. Likewise, in pulmonary alveolar proteinosis (PAP), a rare autoimmune disease characterized by an impaired alveolar macrophage function and a shortness of breath, similar observations have been made. The disease is characterized by the presence of autoantibodies against GM-CSF, in which the reduced bioavailability of GM-CSF is causing an impaired alveolar macrophage development and function [37]. Analyses of several patient-derived autoantibodies reverted to germline, revealed that binding to GM-CSF was strongly diminished or undetectable, indicating that somatic mutations critically determined the specificity to the autoantigen [38]. Likewise, and in concordance with the autoimmune diseases described above, this diminished/loss of antigen recognition after germline reversion has also been described for ACPA, the specific autoantibodies present in patients with RA [39–41]. However, ACPA IgG expressing B cells are unique as they harbor, on average, an extraordinary amount of mutations compared to other (auto)antigen-specific B cells such as anti-Tetanus Toxoid B cells, anti-DSG3, or anti-DNA antibodies [36, 42, 43]. The high mutation load is, possibly, a consequence of the propensity of ACPA to cross-react with several posttranslational modifications and modified antigens [40, 44–47], the putative chronic presence of these antigens and repetitive GC-reactions in the absence of affinity maturation. The high mutation rate complicates the determination of the correct germline sequence, increasing the chance that not the original germline sequence is investigated. In this case, investigating ACPA IgM might be more suitable as these are likely to harbor less mutations. The loss of autoreactivity after germline conversion of autoreactive IgG has also been noted in other rheumatic autoimmune diseases. For example, the analyses of a large set of monoclonal antibodies (mAbs), generated from BCR sequences of IgG memory B cells from Sjögren's syndrome or SLE patients, revealed that a substantial proportion was reactive towards extractable nuclear (auto)antigens (ENA) Ro52 and La. However, when reverted to their unmutated ancestor, the ENA antibodies showed polyreactivity with a low, non-specific, binding to Ro52 supporting the idea that SHM contributed to antibody specificity and introduction of autoreactivity [48, 49]. Similar phenomena have been described in SLE in studies where two dsDNA-directed autoantibodies did not show self-reactivity anymore when expressed in their germline configuration [43,50]. Nevertheless, SHM is certainly not exclusively responsible for self-reactivity in these diseases, as it has also been shown that specific Ro52 reactivity is maintained after reverting somatic mutations of IgG autoantibodies to germline configuration [51].

Thus, several lines of evidence indicate that autoreactivity in certain B cell mediated diseases might be gained by SHM, although additional checkpoints at the postmutational state are likely to be present. However at this stage, it is unknown why SHM that results in an increased avidity of most pathogen-specific responses could also result in autoimmunity and a putative drift away from the initial T cell help involved in the response. A possible explanation could be cross-reactivity of BCRs, as discussed in the next section.

Environmental or microbial cross-reactivity as an inducer of autoimmunity

The observations described above clearly point to the possibility that autoreactive B cell responses emerge from SHM and aberrant subsequent selection of memory B cells. Although observed in several disorders, the prevalence of this mechanism is unclear as this selection process is controlled by other mechanisms as well. For example, B cells typically require the provision of “help” from follicular T helper cells directed against the same antigenic moiety as the responding B cell. In the case of autoantigen recognition as a consequence of SHM to another—unrelated—antigen, the B cells will be severed from T cell help due to the lack of accompanying autoreactive T cells. Therefore, such B cell responses will not be sustained and likely disappear [52, 53]. In this respect, it is remarkable to note that many autoreactive humoral responses are directed against antigens that are tightly linked to RNA or DNA. One of these autoreactive responses is SLE, which is characterized, among others, by autoantibodies directed against ribonucleoproteins (RNP), such as anti-Ro (or anti SS-A), anti-La (or anti-SS-B), or anti-Sm [54]. Similarly, anti-RNP and/or anti-nuclear autoantibodies are found in a variety of other autoimmune diseases such as scleroderma (anti-topoisomerase I; anti-centromere), Sjogren syndrome (anti-SS-A/B), or dermatomyositis (anti-Jo1 recognizing histidine-tRNA ligase) [55–57]. Thus, all these autoantibodies recognize proteins that are intimately linked to DNA/RNA, both able to trigger TLRs. Antigen recognition by such autoreactive B cells will lead to the concurrent provision of TLR7 or TLR9-mediated co-stimulatory signals, hence allowing effective initial activation of these B cells. Intriguingly, highly similar proteins to which these autoreactive B cells react to, are also expressed by microbes. As many DNA- and RNA-binding proteins are highly conserved across species, it is plausible that autoreactive B cells attract “help” from T cells directed against homologous DNA/RNA-binding proteins expressed by microbes. Noteworthy, orthologs of Ro60 with a high sequence similarity to human Ro60 can be found in multiple bacteria, including species of *Corynebacterium*, *Propionibacterium* and *Bacteroides*, which are present in the human skin, oral, and gut microbiota [58]. Thus, individuals chronically colonized by commensal bacteria's expressing Ro60 orthologs, might develop antibodies against both the bacterial and human Ro60 leading to B cell-mediated autoimmunity via cross-reactivity [59]. In this way, the responding B cell could secure the provision of T cell “help” by attracting microbe-specific T cells. Indeed, it has been shown that Ro60 ortholog-containing bacteria are commonly present in the human microbiome and that lupus patients with anti-Ro60 autoantibodies harbor B cell responses to bacterial Ro60 orthologs in vitro [59]. Moreover, it has been shown that the persistent presence of Ro60 orthologs repetitively stimulates short-lived Ro60-specific B cells leading to a sustained autoantibody production and a chronic disease course [60]. These experimental evidences point to the notion that B cell cross-reactivity to conserved epitopes expressed by bacterial- and human DNA/RNA-binding proteins might be central in the breach of peripheral “tolerance” checkpoints in lupus [61].

Similarly, convincing evidence for a “microbe-autoreactive B cell-axis” has been presented recently in the case of the systemic autoimmune disorder antiphospholipid syndrome (APS) [62]. APS is characterized by a well-defined B cell autoantigen, β_2 -glycoprotein I (β_2 GPI) [63]. It has been shown *in vitro* and *in vivo* that anti- β_2 GPI B cells cross-react with mimotopes from the human gut commensal *Roseburia intestinalis*, expressed by the bacterial DNA methyltransferase (DNMT). APS patients express high levels of anti-*Roseburia intestinalis* DNMT IgG antibodies, which correlate with the anti- β_2 GPI antibody levels [62]. Additionally, immunizing mice with *Roseburia intestinalis* resulted in the generation of autoantibodies directed against human β_2 GPI, showing that bacterial proteins, conserved between man and microbe, can provide continued T cell help and hence induce autoimmunity [62]. In these cases, the translocation of microbes to lymph nodes or systemic organs as a result of mucosal barrier breakdown [64], might be at the start of autoimmunity. Translocating bacteria are not only likely presenting antigens to the adaptive immune system but will also activate complement and other innate immune triggers, allowing efficient antigen-presentation to B cells by FDCs as well as co-stimulatory signals for the efficient initiation of B cell responses.

Although DNA/RNA-binding proteins are involved in the examples mentioned above, similar principles are also implicated for other autoimmune responses directed against different self-antigens. Celiac disease, although not primarily a B cell-mediated autoimmune disease, is characterized by disease-specific autoantibodies against the enzyme tissue transglutaminase (tTG) [65]. It is now evident that the T cells underlying the autoreactive tTG-specific B cell response are directed against gliadin, a foreign antigen present in food. tTG can catalyse a specific deamidation of certain glutamine residues in gliadin, leading to the creation of modified gliadin peptides able to bind to the human leukocyte antigen (HLA)-DQ-molecules predisposing to disease and that are recognized by the disease-causing gliadin-reactive T-cells. tTG can form complexes with gliadin and through the uptake of tTG-gliadin complexes, autoreactive tTG-specific B cells can recruit T cell help through an HLA-DQ-restricted presentation of modified gliadin epitopes [66]. Recently, several bacterial mimics of gliadin-epitopes have been identified that were recognized by gluten-reactive T cells from celiac disease patients. Intriguingly, the analyses of crystal structures of T cell receptors derived from gliadin-reactive T cells in complex with HLA-DQ bound to two distinct bacterial peptides, derived from *Pfluorescens*, demonstrated that molecular mimicry drives cross-reactivity toward the gliadin epitopes [67]. Thus, also in the case of celiac disease, it is indicated that T cell reactivity towards microbial antigens displaying cross-reactivity to an antigen present in food, is at play in disease development and the formation of autoreactive B cell responses to an ubiquitously expressed self-antigen.

However, next to microbial antigens, also other environmental triggers might provide T cell help to support autoreactive B cell responses. This is best exemplified for endemic pemphigus foliaceus in Brazil [68]. Pemphigus foliaceus is known worldwide, but an endemic variety, Fogo Selvagem, characterized by the pres-

ence of autoantibodies against DSG1, is only present in certain areas of Brazil [69]. In these areas the unique combination of predisposing HLA-molecules expressed by the population [70] and a well-defined environmental trigger, the bite of a sand fly, has been shown to lead to the formation of anti-DSG1 antibodies [68]. This is explained by the observation that the Salivary protein LJM11 from the sand fly is recognized by Fogo Selvagem antibodies as well as anti-DSG1 mAbs derived from Fogo Selvagem patients. Although the underlying T cell response has not yet been defined, it is highly conceivable that also in this case the autoreactive B cell response arises through cross-reactivity via the “help” of T cells directed against environmental antigens.

Thus, together, the picture emerges that B cell autoimmunity often arises in the absence of autoreactive T cells. Instead, these B cells contain BCRs showing cross-reactivity between self-antigens and environmental/ microbiome triggers and thereby recruit T cell help.

Mechanisms of ACPA expressing B cells in RA to overrun B-cell checkpoints

As described above, the prominent human autoimmune disease RA is characterized by disease-specific autoantibodies and responsive to B cell-targeted therapies. It is manifested by synovial inflammation and progressive joint damage if left untreated. The autoantibody response in RA has been characterized in great detail in the past decade, revealing important insights that harmonize with the findings into the emergence of the autoreactive B cell response uncovered in other autoimmune diseases. However, these studies also unveiled additional processes that are likely involved in violating checkpoints controlling B cell activation in humans. The most disease-specific antibodies in RA are directed against citrullinated proteins, and hence, are named anti-citrullinated protein antibodies. Remarkably, ACPA are reactive towards a broad spectrum of citrullinated antigens, including self-proteins as well as proteins present in microbes [71–75]. Moreover, recent studies have shown that they are not only restricted to citrulline recognition, but that they can also cross-react to other post translational modifications (PTMs), more specifically acetylated- and carbamylated-lysine residues. This has not only been shown at the polyclonal and monoclonal autoantibody level, but also on the level of BCR-signaling [40, 44–46, 76–78]. Thus, in this case, autoreactive B cells expressing a BCR directed against one particular PTM-antigen can not only be activated by this specific modification, but also by (other) antigens expressing different modifications [45]. Hence, various antigens and PTMs could potentially drive the expansion of autoreactive B cells in RA, possibly including microbe-derived antigens. Experimental studies have reflected on a role for the oral, gut or lung microbiota in the development of RA, such as mediated by *Porphyromonas gingivalis* (*Pg*), *Aggregatibacter actinomycetemcomitans* (*Aa*), and *Proteus mirabilis* (*Pm*) [74,79,80]. For example, a high similarity between *Pg* enolase and the human α -enolase has been shown [74]. This similarity between foreign and self allows cross-reactive

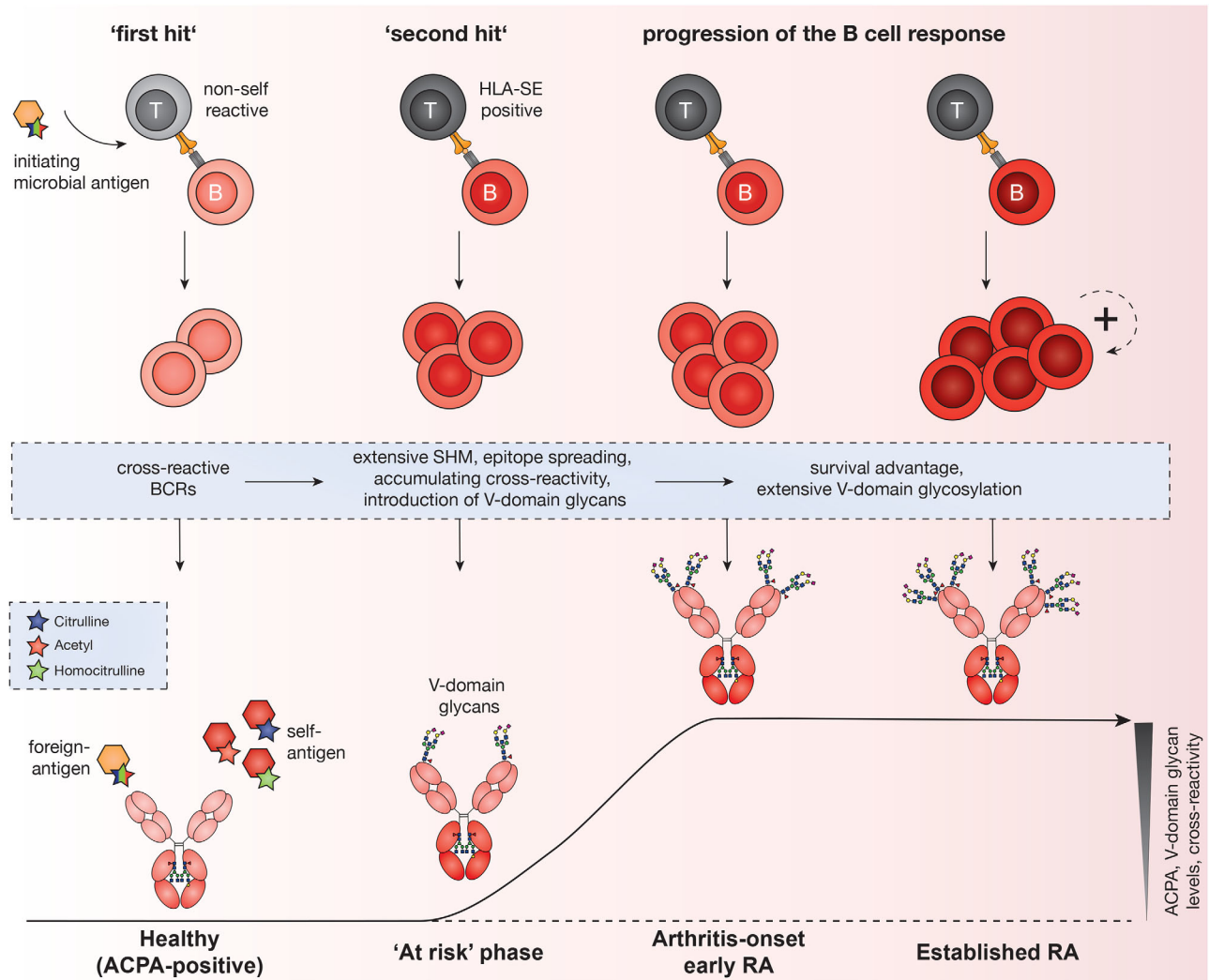


Figure 2. Escape mechanisms of ACPA expressing B cells and RA development. “Multiple hit” theory of the ACPA expressing B cell response in RA and changes in ACPA characteristics toward disease-onset. The autoreactive B cell response in RA is initiated by microbial triggers allowing T cell help and the generation of cross-reactive, citrullinated protein directed B cells and autoantibodies (ACPAs). These B cells are able to recognize post-translationally modified (citrullinated, acetylated, homocitrullinated) foreign- and self-antigens. This ‘first hit’ is followed by a ‘second hit’, including T cells restricted to HLA-molecules predisposing to RA, and results in extensive SHM leading to epitope spreading and an increase in cross-reactivity of the B cell response. This stage is also characterized by the introduction of glycans into the V-domain of the B cell receptors (BCRs). Current epidemiological evidence indicates that these V-domain glycans provide a selective advantage to ACPA-expressing B cells explaining the increase of V-domain-glycosylated ACPA pre-disease and the abundant presence of V-domain glycosylated ACPA in RA. B cells presenting a cross-reactive, highly V-domain glycosylated BCR are probably able to escape important gatekeepers, which will lead to a progression of the B cell response, a rise in autoantibody/ ACPA levels, and thus in the onset of RA.

antibodies to bind to self-antigens, as evidenced by the correlation between ACPA levels directed against citrullinated human α -enolase and the levels of antibodies directed against citrullinated α -enolase from *Pgingivalis* [74]. Additionally, it has been reported that ACPAs might evolve from immune responses to Pg [81]. For *Aggregatibacter actinomycetemcomitans*, it has been suggested that a pore-forming toxin, leukotoxin A (LtxA), causes hypercitrullination and thus generates multiple citrullinated epitopes that can potentially be targeted by the cross-reactive autoantibodies [80]. Nevertheless, ACPA are not only present in individuals exposed to Aa [82]. Thus, also in this autoimmune disease, the prevailing humoral autoimmune response is recog-

nizing both (modified) self- and non-self proteins expressed by microbes. Moreover, these autoantibodies also recognize DNA-binding molecules such as histones as citrullination and acetylation represent prominent epigenomic modifications [83]. Therefore, it is conceivable that ACPA-expressing B cells can recruit co-stimulatory signals from both (opsonized) microbes as well as (modified)protein-DNA-complexes.

Additionally, a unique feature of ACPA is the presence of N-linked glycans in the V-domain as it is found that over 90% of ACPA molecules in serum of RA patients contain V-domain glycans [84,85]. These glycans displayed on ACPA are primarily complex-type carbohydrates containing a high degree of sialic

acids [84] and are acquired through the introduction of *N*-linked glycosylation sites following SHM [86]. As the introduction of glycosylation-sites in the BCR by SHM is a random process, and as ACPA V-domain glycans are found in the vast majority of ACPA molecules in almost all RA-patients, it is likely that the expression of such glycans by anti-citrullinated protein BCRs, provides a selective advantage to ACPA-expressing B cells. Interestingly, the presence of V-domain glycans in ACPA-positive healthy subjects is associated with the transition towards disease [87]. These observations, together with the recent discovery that the HLA-molecules predisposing to RA are associated with the introduction of ACPA V-domain glycans [88], further emphasizes that the introduction of glycans into the BCR V-domain likely represents an additional mechanism involved in the “breach of B cell tolerance” in RA and possibly other human autoimmune diseases including anti-nuclear antibody (ANA)-associated vasculitis and Sjögren’s syndrome [89,90]. How V-domain glycans expressed by autoreactive anti-citrullinated protein-directed B cells contribute to the development and/or expansion of the ACPA-response is presently unclear, although V-domain glycans have been shown to modulate antigen-recognition and to interact with lectins in vicinity to the BCR or on neighboring cells and thereby could deliver survival signals [91–95].

Thus, collectively, autoreactive B cell responses that hallmark RA are of a highly cross-reactive nature, recognizing different PTMs that are expressed on foreign and self-proteins. Their development encompasses the introduction of V-domain glycans through the formation of *N*-linked glycosylation-sites by SHM. Both characteristics are likely to be involved in overrunning B cell checkpoints as cross-reactivity could trigger T cell help as a “first hit” [96], whereas the introduction of V-domain glycans might be an additional “second hit” mechanism to gain a selective activation/survival advantage despite autoreactivity. Autoreactive B cell responses in RA might therefore emerge from ‘multiple hits’, including the high cross-reactive nature of ACPAs and their abundant presence of V-domain glycans (Fig. 2).

Concluding remarks

The mechanisms described in this review shed light on how autoreactive B cells might breach “tolerance” in humans. It is probable that autoreactivity arises in the periphery where B cells have to escape fewer “tolerance” checkpoints than autoreactive B cells emerging in the BM. As the induction of B cell responses in the periphery is stringently controlled by co-stimulation from innate triggers, FDCs and T helper cells and as most autoantibodies have undergone class switch recombination and SHM, it is likely that autoreactive B cell responses arise by misleading these gatekeepers.

Accumulating evidence suggests, although not consistent throughout diseases, that autoreactivity might be gained during SHM allowing mutated BCRs to obtain reactivity to self-antigens. SHM might also introduce cross-reactivity, which allows the BCRs to react towards both self- and foreign antigens. Cross-reactivity

to non-self as an inducer of autoimmunity is highlighted in several studies demonstrating an important role for the microbiome or environmental triggers in the induction of B cell mediated autoimmune responses. A common denominator of these findings is the notion that autoreactive B cells cross-react between self- and the “environment”/microbiome, whereas the accommodating T cells supporting these B cells do not necessarily recognize self. In this way, the need to depose both B cell and T cell checkpoints to induce B cell-mediated autoimmunity is overturned, conceivably explaining the relative high prevalence of B cell-mediated autoimmune responses in the human population. Additionally, glycans present in the V-domain of autoreactive BCRs might provide an additional, still poorly studied, mechanism involved in the induction of autoimmunity in humans. Further understanding of these, and other pathways controlling the induction of autoreactive B cell responses in humans, is crucial for the development of preventative and/or curative strategies in B cell-mediated autoimmune diseases; diseases that are still incurable to date.

Acknowledgements: This work has been financially supported by ReumaNederland (17-1-402), the IMI funded project RTCure (777357), the Netherlands Organization for Scientific Research NWO (91214031) and has been funded as part of the Target-to-B! consortium by ‘Samenwerkende Gezondheidsfondsen’ (SGF) consisting of 20 health funds including KWF Kankerbestrijding, ReumaNederland, Topsector Life Sciences & Health (Health~Holland) and the Business Life (LSHM18055-SGF). R.E.M.T. is the recipient of an European Research Council (ERC) Advanced grant (AdG2019-884796).

Author contributions: All authors were involved in designing the Figures, drafting the article and revising it critically. All authors approved the final version to be published.

Conflict of interest: REMT is named as co-inventor on a patent on ACPA-IgG V-domain glycosylation. All other authors declare no commercial or financial conflict of interests.

References

- 1 Edwards, J. C. and Cambridge, G., Prospects for B-cell-targeted therapy in autoimmune disease. *Rheumatology (Oxford)* 2005. **44**: 151–156.
- 2 Edwards, J. C. W., Szczepański, L., Szechiński, J., Filipowicz-Sosnowska, A., Emery, P., Close, D. R., Stevens, R. M. and Shaw, T., Efficacy of B-Cell-Targeted Therapy with Rituximab in Patients with Rheumatoid Arthritis. *N. Engl. J. Med.* 2004. **350**: 2572–2581.
- 3 Joly, P., Maho-Vaillant, M., Prost-Squarcioni, C., Hebert, V., Houivet, E., Calbo, S., Caillot, F. et al., First-line rituximab combined with short-term prednisone versus prednisone alone for the treatment of pemphigus (Ritux 3): a prospective, multicentre, parallel-group, open-label randomised trial. *Lancet North Am. Ed.* 2017. **389**: 2031–2040.

- 4 Musette, P. and Bouaziz, J. D., B cell modulation strategies in autoimmune diseases: new concepts. *Front. Immunol.* 2018.
- 5 Hampe, C. S., B Cell in Autoimmune Diseases. *Scientifica (Cairo)* 2012. 2012.
- 6 Hofmann, K., Clauder, A.-K. and Manz, R. A., Targeting B cells and plasma cells in autoimmune diseases. *Front. Immunol.* 2018. 9.
- 7 Tonegawa, S., Somatic generation of antibody diversity. *Nature* 1983. 302: 575–581.
- 8 Lewis, S., Gifford, A. and Baltimore, D., DNA elements are asymmetrically joined during the site-specific recombination of kappa immunoglobulin genes. *Science* 1985. 228: 677–685.
- 9 Lewis, S., Gifford, A. and Baltimore, D., Joining of V kappa to J kappa gene segments in a retroviral vector introduced into lymphoid cells. *Nature* 1984. 308: 425–428.
- 10 Meffre, E. and Wardemann, H., B-cell tolerance checkpoints in health and autoimmunity. *Curr. Opin. Immunol.* 2008. 20: 632–638.
- 11 Meffre, E., The establishment of early B cell tolerance in humans: lessons from primary immunodeficiency diseases. *Ann. N. Y. Acad. Sci.* 2011. 1246: 1–10.
- 12 Grandien, A., Fuchs, R., Nobrega, A., Andersson, J. and Coutinho, A., Negative selection of multireactive B cell clones in normal adult mice. *Eur. J. Immunol.* 1994. 24: 1345–1352.
- 13 Hikida, M. and Ohmori, H., Rearrangement of lambda light chain genes in mature B cells in vitro and in vivo. Function of reexpressed recombination-activating gene (RAG) products. *J. Exp. Med.* 1998. 187: 795–799.
- 14 Cyster, J. G. and Goodnow, C. C., Antigen-induced exclusion from follicles and anergy are separate and complementary processes that influence peripheral B cell fate. *Immunity* 1995. 3: 691–701.
- 15 Cyster, J. G., Hartley, S. B. and Goodnow, C. C., Competition for follicular niches excludes self-reactive cells from the recirculating B-cell repertoire. *Nature* 1994. 371: 389–395.
- 16 Allen, C. D. and Cyster, J. G., Follicular dendritic cell networks of primary follicles and germinal centers: phenotype and function. *Semin. Immunol.* 2008. 20: 14–25.
- 17 MacLennan, I. C. Germinal centers. *Annu. Rev. Immunol.* 1994. 12: 117–139.
- 18 Mandel, T. E., Phipps, R. P., Abbot, A. P. and Tew, J. G., Long-term antigen retention by dendritic cells in the popliteal lymph node of immunized mice. *Immunology* 1981. 43: 353–362.
- 19 Goodnow, C. C., Brink, R. and Adams, E., Breakdown of self-tolerance in anergic B lymphocytes. *Nature* 1991. 352: 532–536.
- 20 Ansel, K. M., Ngo, V. N., Hyman, P. L., Luther, S. A., Forster, R., Sedgwick, J. D., Browning, J. L., Lipp, M. and Cyster, J. G., A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature* 2000. 406: 309–314.
- 21 Wu, Y., El Shikh, M. E., El Sayed, R. M., Best, A. M., Szakal, A. K. and Tew, J. G., IL-6 produced by immune complex-activated follicular dendritic cells promotes germinal center reactions, IgG responses and somatic hypermutation. *Int. Immunol.* 2009. 21: 745–756.
- 22 Heesters, B. A., Chatterjee, P., Kim, Y. A., Gonzalez, S. F., Kuligowski, M. P., Kirchhausen, T. and Carroll, M. C., Endocytosis and recycling of immune complexes by follicular dendritic cells enhances B cell antigen binding and activation. *Immunity* 2013. 38: 1164–1175.
- 23 Suzuki, K., Maruya, M., Kawamoto, S., Sitnik, K., Kitamura, H., Agace, W. W. and Fagarasan, S., The sensing of environmental stimuli by follicular dendritic cells promotes immunoglobulin A generation in the gut. *Immunity* 2010. 33: 71–83.
- 24 Fischer, M. B., Goerg, S., Shen, L., Prodeus, A. P., Goodnow, C. C., Kelsoe, G. and Carroll, M. C., Dependence of germinal center B cells on expression of CD21/CD35 for survival. *Science* 1998. 280: 582–585.
- 25 Roozendaal, R. and Carroll, M. C., Complement receptors CD21 and CD35 in humoral immunity. *Immunol. Rev.* 2007. 219: 157–166.
- 26 Yau, I. W., Cato, M. H., Jellusova, J., Hurtado de Mendoza, T., Brink, R. and Rickert, R. C., Censoring of self-reactive B cells by follicular dendritic cell-displayed self-antigen. *J. Immunol.* 2013. 191: 1082–1090.
- 27 Hanayama, R., Tanaka, M., Miyasaka, K., Aozasa, K., Koike, M., Uchiyama, Y. and Nagata, S., Autoimmune disease and impaired uptake of apoptotic cells in MFG-E8-deficient mice. *Science* 2004. 304: 1147–1150.
- 28 Baumann, I., Kolowos, W., Voll, R. E., Manger, B., Gaip, U., Neuhuber, W. L., Kirchner, T. et al., Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus. *Arthritis Rheum.* 2002. 46: 191–201.
- 29 Victoratos, P. and Kollias, G., Induction of autoantibody-mediated spontaneous arthritis critically depends on follicular dendritic cells. *Immunity* 2009. 30: 130–142.
- 30 Humby, F., Bombardieri, M., Manzo, A., Kelly, S., Blades, M. C., Kirkham, B., Spencer, J. and Pitzalis, C., Ectopic lymphoid structures support ongoing production of class-switched autoantibodies in rheumatoid synovium. *PLoS Med.* 2009. 6: e1.
- 31 Randen, I., Mellbye, O. J., Forre, O. and Natvig, J. B., The identification of germinal centres and follicular dendritic cell networks in rheumatoid synovial tissue. *Scand. J. Immunol.* 1995. 41: 481–486.
- 32 Reed, J. H., Jackson, J., Christ, D. and Goodnow, C. C., Clonal redemption of autoantibodies by somatic hypermutation away from self-reactivity during human immunization. *J. Exp. Med.* 2016. 213: 1255–1265.
- 33 Berkowitz, P., Chua, M., Liu, Z., Diaz, L. A. and Rubenstein, D. S., Autoantibodies in the autoimmune disease pemphigus foliaceus induce blistering via p38 mitogen-activated protein kinase-dependent signaling in the skin. *Am. J. Pathol.* 2008. 173: 1628–1636.
- 34 Amagai, M., Hashimoto, T., Shimizu, N. and Nishikawa, T., Absorption of pathogenic autoantibodies by the extracellular domain of pemphigus vulgaris antigen (Dsg3) produced by baculovirus. *J. Clin. Invest.* 1994. 94: 59–67.
- 35 Amagai, M., Klaus-Kovtun, V. and Stanley, J. R., Autoantibodies against a novel epithelial cadherin in pemphigus vulgaris, a disease of cell adhesion. *Cell* 1991. 67: 869–877.
- 36 Di Zenzo, G., Di Lullo, G., Corti, D., Calabresi, V., Sinistro, A., Vanzetta, F., Didona, B. et al., Pemphigus autoantibodies generated through somatic mutations target the desmoglein-3 cis-interface. *J. Clin. Invest.* 2012. 122: 3781–3790.
- 37 Kitamura, T., Tanaka, N., Watanabe, J., Uchida Kanegasaki, S., Yamada, Y. and Nakata, K., Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* 1999. 190: 875–880.
- 38 Piccoli, L., Campo, I., Fregni, C. S., Rodriguez, B. M. F., Minola, A., Sallusto, F., Luisetti, M. et al., Neutralization and clearance of GM-CSF by autoantibodies in pulmonary alveolar proteinosis. *Nat. Commun.* 2015. 6: 7375.
- 39 Kongpachith, S., Lingampalli, N., Ju, C. H., Blum, L. K., Lu, D. R., Elliott, S. E., Mao, R. et al., Affinity maturation of the anti-citrullinated protein antibody paratope drives epitope spreading and polyreactivity in rheumatoid arthritis. *Arthritis & Rheumatology* 2019. 71: 507–517.
- 40 Steen, J., Forsstrom, B., Sahlstrom, P., Odowd, V., Israelsson, L., Krishnamurthy, A., Badreh, S. et al., Recognition of amino acid motifs, rather than specific proteins, by human plasma cell-derived monoclonal antibodies to posttranslationally modified proteins in rheumatoid arthritis. *Arthritis Rheumatol* 2019. 71: 196–209.
- 41 Ozawa, T., Ouhara, K., Tsuda, R., Munenaga, S., Kurihara, H., Kohno, H., Hamana, H. et al., Physiological target, molecular evolution and

- pathogenic functions of a monoclonal ACPA obtained from an RA patient. *Arthritis & Rheumatology* 2020.
- 42 Vergoosen, R. D., Slot, L. M., Hafkenscheid, L., Koning, M. T., Van Der Voort, E. I. H., Grooff, C. A., Zervakis, G. et al., B-cell receptor sequencing of anti-citrullinated protein antibody (ACPA) IgG-expressing B cells indicates a selective advantage for the introduction of N-glycosylation sites during somatic hypermutation. *Ann. Rheum. Dis.* 2017: 201.
- 43 Sakakibara, S., Arimori, T., Yamashita, K., Jinzai, H., Motooka, D., Nakamura, S., Li, S. et al., Clonal evolution and antigen recognition of anti-nuclear antibodies in acute systemic lupus erythematosus. *Sci. Rep.* 2017..
- 44 Kampstra, A. S. B., Dekkers, J. S., Volkov, M., Dorjee, A. L., Hafkenscheid, L., Kempers, A. C., van Delft, M. et al., Different classes of anti-modified protein antibodies are induced on exposure to antigens expressing only one type of modification. *Ann. Rheum. Dis.* 2019. 78: 908–916.
- 45 Kissel, T., Reijm, S., Slot, L. M., Cavallari, M., Wortel, C. M., Vergoosen, R. D., Stoeken-Rijsbergen, G. et al., Antibodies and B cells recognising citrullinated proteins display a broad cross-reactivity towards other post-translational modifications. *Ann. Rheum. Dis.* 2020. 79: 472–480.
- 46 Reed, E., Jiang, X., Kharlamova, N., Ytterberg, A. J., Catrina, A. I., Israelsson, L., Mathsson-Alm, L. et al., Antibodies to carbamylated alpha-enolase epitopes in rheumatoid arthritis also bind citrullinated epitopes and are largely indistinct from anti-citrullinated protein antibodies. *Arthritis Res. Ther.* 2016. 18: 96.
- 47 Figueiredo, C. P., Bang, H., Cobra, J. F., Englbrecht, M., Hueber, A. J., Haschka, J., Manger, B. et al., Antimodified protein antibody response pattern influences the risk for disease relapse in patients with rheumatoid arthritis tapering disease modifying antirheumatic drugs. *Ann. Rheum. Dis.* 2017. 76: 399–407.
- 48 Mietzner, B., Tsujii, M., Scheid, J., Velinzon, K., Tiller, T., Abraham, K., Gonzalez, J. B. et al., Autoreactive IgG memory antibodies in patients with systemic lupus erythematosus arise from nonreactive and polyreactive precursors. *Proc. Natl. Acad. Sci.* 2008. 105: 9727–9732.
- 49 Takeshita, M., Suzuki, K., Kaneda, Y., Yamane, H., Ikeura, K., Sato, H., Kato, S. et al., Antigen-driven selection of antibodies against SSA, SSB and the centromere ‘complex’, including a novel antigen, MIS12 complex, in human salivary glands. *Ann. Rheum. Dis.* 2020. 79: 150–158.
- 50 Wellmann, U., Letz, M., Herrmann, M., Angermuller, S., Kalden, J. R. and Winkler, T. H. The evolution of human anti-double-stranded DNA autoantibodies 2005. 102: 9258–9263.
- 51 Reed, J. H., Gorny, M. K., Li, L., Cardozo, T., Buyon, J. P. and Clancy, R. M., Ro52 autoantibodies arise from self-reactive progenitors in a mother of a child with neonatal lupus. *J. Autoimmun.* 2017. 79: 99–104.
- 52 Mesin, L., Ersching, J. and Victora, G. D., Germinal Center B Cell Dynamics. *Immunity* 2016. 45: 471–482.
- 53 Schwickert, T. A., Victora, G. D., Fooksman, D. R., Kamphorst, A. O., Mugnier, M. R., Gitlin, A. D., Dustin, M. L. et al., A dynamic T cell-limited checkpoint regulates affinity-dependent B cell entry into the germinal center. *J. Exp. Med.* 2011. 208: 1243–1252.
- 54 Dema, B. and Charles, N., Autoantibodies in SLE: Specificities, Isotypes and Receptors. *Antibodies (Basel)* 2016. 5.
- 55 Stochmal, A., Czuwara, J., Trojanowska, M. and Rudnicka, L., Antinuclear antibodies in systemic sclerosis: an update. *Clin. Rev. Allergy Immunol.* 2020. 58: 40–51.
- 56 Fayyaz, A., Kurien, B. T. and Scofield, R. H., Autoantibodies in Sjögren's Syndrome. *Rheumatic Disease Clinics of North America* 2016. 42: 419–434.
- 57 Mileti, L. M., Strek, M. E., Niewold, T. B., Curran, J. J. and Sweiss, N. J., Clinical characteristics of patients with anti-jo-1 antibodies. *JCR: Journal of Clinical Rheumatology* 2009. 15: 254–255.
- 58 Sim, S. and Wolin, S. L., Emerging roles for the Ro 60-kDa autoantigen in noncoding RNA metabolism. *Wiley Interdiscip Rev RNA* 2011. 2: 686–699.
- 59 Greiling, T. M., Dehner, C., Chen, X., Hughes, K., Iniguez, A. J., Boccitto, M., Ruiz, D. Z. et al., Commensal orthologs of the human autoantigen Ro60 as triggers of autoimmunity in lupus. *Sci. Transl. Med.* 2018..
- 60 Lindop, R., Arentz, G., Bastian, I., Whyte, A. F., Thurgood, L. A., Chataway, T. K., Jackson, M. W. et al., Long-term Ro60 humoral autoimmunity in primary Sjogren's syndrome is maintained by rapid clonal turnover. *Clin. Immunol.* 2013. 148: 27–34.
- 61 McClain, M. T., Heinlen, L. D., Dennis, G. J., Roebuck, J., Harley, J. B. and James, J. A., Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry. *Nat. Med.* 2005. 11: 85–89.
- 62 Ruff, W. E., Dehner, C., Kim, W. J., Pagovich, O., Aguiar, C. L., Yu, A. T., Roth, A. S. et al., Pathogenic autoreactive T and B cells cross-react with mimotopes expressed by a common human gut commensal to trigger autoimmunity. *Cell Host Microbe* 2019. 26: 100–113.
- 63 Ruiz-Irastorza, G., Crowther, M., Branch, W. and Khamashta, M. A., Antiphospholipid syndrome. *Lancet* 2010. 376: 1498–1509.
- 64 Manfredo Vieira, S., Hiltensperger, M., Kumar, V., Zegarra-Ruiz, D., Dehner, C., Khan, N., Costa, F. R. C. et al., Translocation of a gut pathobiont drives autoimmunity in mice and humans. *Science* 2018. 359: 1156–1161.
- 65 Caja, S., Mäki, M., Kaukinen, K. and Lindfors, K., Antibodies in celiac disease: implications beyond diagnostics. *Cellular & Molecular Immunology* 2011. 8: 103–109.
- 66 Caio, G., Volta, U., Sapone, A., Leffler, D. A., De Giorgio, R., Catassi, C. and Fasano, A., Celiac disease: a comprehensive current review. *BMC Medicine* 2019. 17.
- 67 Petersen, J., Ciacchi, L., Tran, M. T., Loh, K. L., Kooy-Winkelaar, Y., Croft, N. P., Hardy, M. Y. et al., T cell receptor cross-reactivity between gliadin and bacterial peptides in celiac disease. *Nat. Struct. Mol. Biol.* 2020. 27: 49–61.
- 68 Qian, Y., Jeong, J. S., Maldonado, M., Valenzuela, J. G., Gomes, R., Teixeira, C., Evangelista, F. et al., Cutting edge: Brazilian pemphigus foliaceus anti-desmoglein 1 autoantibodies cross-react with sand fly salivary. *LJM11 Antigen* 2012. 189: 1535–1539.
- 69 Diaz, L. A., Sampaio, S. A. P., Rivitti, E. A., Martins, C. R., Cunha, P. R., Lombardi, C., Almeida, F. A. et al., Endemic pemphigus foliaceus (Fogo Selvagem): II. *Current and Historic Epidemiologic Studies* 1989. 92: 4–12.
- 70 Moraes, J. R., Moraes, M. E., Fernandez-Vina, M., Diaz, L. A., Friedman, H., Campbell, I. T., Alvarez, R. R. et al., HLA antigens and risk for development of pemphigus foliaceus (fogo selvagem) in endemic areas of Brazil. *Immunogenetics* 1991. 33: 388–391.
- 71 Takizawa, Y., Suzuki, A., Sawada, T., Ohsaka, M., Inoue, T., Yamada, R. and Yamamoto, K., Citrullinated fibrinogen detected as a soluble citrullinated autoantigen in rheumatoid arthritis synovial fluids. *Ann. Rheum. Dis.* 2006. 65: 1013–1020.
- 72 Burkhardt, H., Koller, T., Engstrom, A., Nandakumar, K. S., Turnay, J., Kraetsch, H. G., Kalden, J. R. et al., Epitope-specific recognition of type II collagen by rheumatoid arthritis antibodies is shared with recognition by antibodies that are arthritogenic in collagen-induced arthritis in the mouse. *Arthritis Rheum.* 2002. 46: 2339–2348.
- 73 Vossenaar, E. R., Despres, N., Lapointe, E., van der Heijden, A., Lora, M., Senshu, T., van Venrooij, W. J. et al., Rheumatoid arthritis specific anti-Sa antibodies target citrullinated vimentin. *Arthritis Res. Ther.* 2004. 6: R142–150.
- 74 Lundberg, K., Kinloch, A., Fisher, B. A., Wegner, N., Wait, R., Charles, P., Mikuls, T. R. and Venables, P. J., Antibodies to citrullinated alpha-enolase peptide 1 are specific for rheumatoid arthritis and cross-react with bacterial enolase. *Arthritis Rheum.* 2008. 58: 3009–3019.
- 75 Ioan-Facsinay, A., el-Bannoudi, H., Scherer, H. U., van der Woude, D., Menard, H. A., Lora, M., Trouw, L. A. et al., Anti-cyclic citrullinated peptide antibodies are a collection of anti-citrullinated protein antibodies and contain overlapping and non-overlapping reactivities. *Ann. Rheum. Dis.* 2011. 70: 188–193.

- 76 Lloyd, K. A., Wigerblad, G., Sahlstrom, P., Garimella, M. G., Chemin, K., Steen, J., Titcombe, P. J. et al., Differential ACPA Binding to Nuclear Antigens Reveals a PAD-Independent Pathway and a Distinct Subset of Acetylation Cross-Reactive Autoantibodies in Rheumatoid Arthritis. *Front. Immunol.* 2018. 9: 3033.
- 77 Shi, J., Knevel, R., Suwannalai, P., van der Linden, M. P., Janssen, G. M., van Veelen, P. A., Levarht, N. E. et al., Autoantibodies recognizing carbamylated proteins are present in sera of patients with rheumatoid arthritis and predict joint damage. *Proc. Natl. Acad. Sci. U. S. A.* 2011. 108: 17372–17377.
- 78 Dekkers, J. S., Verheul, M. K., Stoop, J. N., Liu, B., Ioan-Facsinay, A., van Veelen, P. A., de Ru, A. H. et al., Breach of autoreactive B cell tolerance by post-translationally modified proteins. *Ann. Rheum. Dis.* 2017. 76: 1449–1457.
- 79 Rashid, T., Jayakumar, K. S., Binder, A., Ellis, S., Cunningham, P. and Ebringer, A., Rheumatoid arthritis patients have elevated antibodies to cross-reactive and non cross-reactive antigens from Proteus microbes. *Clin. Exp. Rheumatol.* 2007. 25: 259–267.
- 80 Konig, M. F., Abusleme, L., Reinholdt, J., Palmer, R. J., Teles, R. P., Sampson, K., Rosen, A. et al., Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. *Sci. Transl. Med.* 2016. 8: 369ra176.
- 81 Li, S., Yu, Y., Yue, Y., Liao, H., Xie, W., Thai, J., Mikuls, T. R. et al., Autoantibodies From Single Circulating Plasmablasts React With Citrullinated Antigens and Porphyromonas gingivalis in Rheumatoid Arthritis. *Arthritis Rheumatol* 2016. 68: 614–626.
- 82 Volkov, M., Dekkers, J., Loos, B. G., Bizzarro, S., Huizinga, T. W. J., Praetorius, H. A., Toes, R. E. M. et al., Comment on “Aggregatibacter actinomycetemcomitans-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis”. *Sci. Transl. Med.* 2018.
- 83 Dieker, J. and Muller, S., *Epigenetic Histone Code and Autoimmunity* 2010. 39: 78–84.
- 84 Hafkenscheid, L., Bondt, A., Scherer, H. U., Huizinga, T. W., Wuhler, M., Toes, R. E. and Rombouts, Y., Structural Analysis of Variable Domain Glycosylation of Anti-Citrullinated Protein Antibodies in Rheumatoid Arthritis Reveals the Presence of Highly Sialylated Glycans. *Mol. Cell. Proteomics* 2017. 16: 278–287.
- 85 Rombouts, Y., Willemze, A., van Beers, J. J., Shi, J., Kerkman, P. F., van Toorn, L., Janssen, G. M. et al., Extensive glycosylation of ACPA-IgG variable domains modulates binding to citrullinated antigens in rheumatoid arthritis. *Ann. Rheum. Dis.* 2016. 75: 578–585.
- 86 Vergoesen, R. D., Slot, L. M., Hafkenscheid, L., Koning, M. T., van der Voort, E. I. H., Grooff, C. A., Zervakis, G. et al., B-cell receptor sequencing of anti-citrullinated protein antibody (ACPA) IgG-expressing B cells indicates a selective advantage for the introduction of N-glycosylation sites during somatic hypermutation. *Ann. Rheum. Dis.* 2018. 77: 956–958.
- 87 Hafkenscheid, L., de Moel, E., Smolik, I., Tanner, S., Meng, X., Jansen, B. C., Bondt, A. et al., N-Linked Glycans in the Variable Domain of IgG Anti-Citrullinated Protein Antibodies Predict the Development of Rheumatoid Arthritis. *Arthritis Rheumatol* 2019. 71: 1626–1633.
- 88 Kissel, T., van Schie, K. A., Hafkenscheid, L., Lundquist, A., Kokkonen, H., Wuhler, M., Huizinga, T. W., Scherer, H. U., Toes, R. and Rantapaa-Dahlqvist, S., On the presence of HLA-SE alleles and ACPA-IgG variable domain glycosylation in the phase preceding the development of rheumatoid arthritis. *Ann. Rheum. Dis.* 2019. 78: 1616–1620.
- 89 Holland, M., Yagi, H., Takahashi, N., Kato, K., Savage, C. O., Goodall, D. M. and Jefferis, R., Differential glycosylation of polyclonal IgG, IgG-Fc and IgG-Fab isolated from the sera of patients with ANCA-associated systemic vasculitis. *Biochim. Biophys. Acta* 2006. 1760: 669–677.
- 90 Hamza, N., Hershberg, U., Kallenberg, C. G., Vissink, A., Spijkervet, F. K., Bootsma, H., Kroese, F. G. et al., Ig gene analysis reveals altered selective pressures on Ig-producing cells in parotid glands of primary Sjogren's syndrome patients. *J. Immunol.* 2015. 194: 514–521.
- 91 van de Bovenkamp, F. S., Derksen, N. I. L., Ooijevaar-de Heer, P., van Schie, K. A., Kruijthof, S., Berkowska, M. A., van der Schoot, C. E. et al., Adaptive antibody diversification through N-linked glycosylation of the immunoglobulin variable region. *Proc. Natl. Acad. Sci. U. S. A.* 2018. 115: 1901–1906.
- 92 Sabouri, Z., Schofield, P., Horikawa, K., Spierings, E., Kipling, D., Randall, K. L., Langley, D. 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.* 2014. 111: E2567–2575.
- 93 Radcliffe, C. M., Arnold, J. N., Suter, D. M., Wormald, M. R., Harvey, D. J., Royle, L., Mimura, Y. et al., Human Follicular Lymphoma Cells Contain Oligomannose Glycans in the Antigen-binding Site of the B-cell Receptor. *J. Biol. Chem.* 2007. 282: 7405–7415.
- 94 Zhu, D., McCarthy, H., Ottensmeier, C. H., Johnson, P., Hamblin, T. J. and Stevenson, F. K., Acquisition of potential N-glycosylation sites in the immunoglobulin variable region by somatic mutation is a distinctive feature of follicular lymphoma. *Blood* 2002. 99: 2562–2568.
- 95 Vletter, E. M., Koning, M. T., Scherer, H. U., Veelken, H. and Toes, R. E. M. A Comparison of Immunoglobulin Variable Region N-Linked Glycosylation in Healthy Donors, Autoimmune Disease and Lymphoma. *Front. Immunol.* 2020. 11.
- 96 van Heemst, J., Jansen, D. T., Polydorides, S., Moustakas, A. K., Bax, M., Feitsma, A. L., Bontrop-Elferink, D. G. et al., Crossreactivity to vinculin and microbes provides a molecular basis for HLA-based protection against rheumatoid arthritis. *Nat. Commun.* 2015. 6: 6681.

Abbreviations: **Aa:** aggregatibacter actinomycetemcomitans · **AAV:** anti-neutrophil cytoplasmic antibody-associated vasculitis · **ACPA:** anti-citrullinated protein antibody · **ANA:** anti-nuclear antibody · **APS:** anti-phospholipid syndrome · **BCR:** B cell receptor · **BM:** bone marrow · **B₂GPI:** B₂-glycoprotein I · **D:** diversity · **DNMT:** DNA methyltransferase · **DSG:** desmoglein · **ENA:** extractable nuclear (auto)antigen · **FDC:** follicular dendritic cell · **GC:** germinal center · **GM-CSF:** granulocyte-macrophage colony-stimulating factor · **HLA:** human leukocyte antigen · **IC:** immune complex · **J:** joining · **LPS:** lipopolysaccharides · **LtxA:** leukotoxin A · **mAb:** monoclonal antibody · **PAP:** pulmonary alveolar proteinosis · **Pg:** porphyromonas gingivalis · **Pm:** proteus mirabilis · **PTM:** post-translational modification · **PV:** phemphigus vulgaris · **RA:** rheumatoid arthritis · **RNP:** ribonucleoproteins · **SHM:** somatic hypermutation · **SLE:** systemic lupus erythematosus · **TLR:** toll-like receptor · **tTG:** tissue transglutaminase · **V:** Variable

Full correspondence: Dr. R.E.M. Toes, Department of Rheumatology, Leiden University Medical Center, Albinusdreef 2, PO Box 9600, 2300 RC Leiden, The Netherlands.
e-mail: R.E.M.Toes@lumc.nl

Received: 17/9/2020
Accepted: 4/11/2020
Accepted article online: 5/11/2020