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B cell receptor repertoire abnormalities in autoimmune disease

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B cells play a crucial role in the immune response and contribute to various autoimmune diseases. Recent studies have revealed abnormalities in the B cell receptor (BCR) repertoire of patients with autoimmune diseases, with distinct features observed among different diseases and B cell subsets. Classically, BCR repertoire was used as an identifier of distinct antigen-specific clonotypes, but the recent advancement of analyzing large-scale repertoire has enabled us to use it as a tool for characterizing cellular biology. In this review, we provide an overview of the BCR repertoire in autoimmune diseases incorporating insights from our latest research findings. In systemic lupus erythematosus (SLE), we observed a significant skew in the usage of VDJ genes, particularly in CD27$^+$IgD$^+$ unswitched memory B cells and plasmablasts. Notably, autoreactive clones within unswitched memory B cells were found to be increased and strongly associated with disease activity, underscoring the clinical significance of this subset. Similarly, various abnormalities in the BCR repertoire have been reported in other autoimmune diseases such as rheumatoid arthritis. Thus, BCR repertoire analysis holds potential for enhancing our understanding of the underlying mechanisms involved in autoimmune diseases. Moreover, it has the potential to predict treatment effects and identify therapeutic targets in autoimmune diseases.

Introduction

B cells are at the core of the adaptive humoral immune system. One of the main features of B cells is to secrete antigen-specific antibody. B cells undergo VDJ gene recombination and somatic hypermutation (SHM) processes to yield a diverse array of B cell receptors (BCRs) enabling recognition of a wide spectrum of antigens (1, 2). The collection of individual BCRs is called the BCR repertoire and reflects the state of the immune system (3,

4). Due to its complexity, it was difficult to obtain information on the exact sequence of the BCR region in a large scale. However, advancements in sequencing technology have facilitated the acquisition of intricate BCR sequence data (5). BCR repertoire analysis has revealed abnormalities in repertoire in patients with autoimmune diseases at the level of B cell subsets, which is useful for understanding the diseases and investigating therapeutic targets. This article summarizes what is currently known about BCR repertoire analysis in autoimmune diseases.

BCR repertoire analysis

BCR constitutes a tetrameric membrane-bound protein composed of two heavy chains and two light chains, each possessing variable and constant regions. Recombination of the genes encoding the variable region (IGHV, IGHD, and IGHJ in the heavy chains and IGLV and IGLJ in the light chains), deletion or insertion of nucleotides in the junction and subsequent SHM create diversity in the structure of the variable region, allowing the production of antibodies to a variety of foreign antigens (6). Within the variable region, three complementarity-determining regions (CDR) directly engage with antigens. Among these, CDR3 displays the utmost diversity and plays the most important role for antigen specificity (7). A comprehensive characterization of BCRs holds the key to a deeper comprehension of the immune landscape.

Recent advancements in high-throughput sequencing technology have revolutionized the accurate reading of BCR sequences (8). Reading the sequence of BCRs can provide information on the characteristics of BCR repertoire from various perspectives. The CDR3 amino acid sequence can be assumed to be unique to each clone, allowing us to estimate the clonality and diversity of BCR repertoire. Also, chemical or physical features of CDR3 sequences, such as length, hydrophobicity and charge, can represent the repertoire property. Gene usage, meaning the relative fraction of V (or D, J) gene in the repertoire, can also reflect its characteristics. By comparing the nucleotide sequences with germline sequence, we can estimate the degree of SHM (9, 10). Each of these features has been associated with infection, immunization and autoimmunity (11, 12).

BCR repertoire abnormalities in autoimmune diseases

Most of autoimmune diseases are characterized by the break of immune tolerance to self-antigens and the emergence of autoantibodies. Thus, identification of autoreactive BCR clone and elucidation of the mechanisms which lead to its emergence has been of great interest in this research field. Studies have mainly focused on the specific autoreactive clones and have successfully identified the disease specific features of autoantibodies (13, 14). Recently, some studies have also tried to characterize the global repertoire abnormality in immune-mediated diseases (15, 16).

Systemic lupus erythematosus (SLE) is a systemic disease that affects a variety of organs including skin, kidney, and central nervous system (17). Although the pathogenesis of SLE has not been fully understood, autoreactive B cells and autoantibodies are thought to be important factors in its pathogenesis. There is a bias in the usage of IGHV genes compared to healthy individuals, including increased usage of the IGHV4 gene family (18, 19). Among IGHV4 family, various lines of evidence support the association of IGHV4-34 gene with disease pathogenesis. First, germline sequence of IGHV4-34 has a property of self-antigen binding and usually eliminated from memory B cell subset by negative selection (20). Second, the quantity of IGHV4-34 antibodies with 9G4 idiotype, defined by conserved germline AVY and QW amino acid motifs in the framework-1 region, is elevated in SLE patients (13). Third, in the longitudinal analysis of SLE patient BCR repertoire, usage of IGHV4-34 was increased in the acute state (21). Also, in our recent study of more than 100 SLE patients, IGHV4-34 usage, especially with 9G4 idiotype, showed significant association with disease activity. Interestingly, prominent association was observed in unswitched memory B (USM B), but not in other memory subsets, suggesting the critical role of USM B in its pathogenesis (16) (also see the next section).

Together with gene usage, CDR3 length has long been used as an index of repertoire skewness. In general, CDR3 length is relatively long in naïve B cells, but such long CDR3 has more autoreactivity and thus negatively selected during its maturation to memory B cells (12). In SLE patients, CDR3 length of class-switched memory B cells and plasmablasts were longer than healthy controls, suggesting the breakdown of peripheral checkpoint (15, 16). On the contrary, CDR3 length of naïve B cells were shorter in SLE patients than healthy controls in our recent study (16). As CDR3 length was also short in non-productive CDR3 sequence and strongly correlated with interferon signature in transcriptome, we hypothesized that high interferon activity in bone marrow could affect the early development of B cells (16). As exemplified here, CDR3 length should allow us some biological interpretation of repertoire abnormality. Besides, cell-type dependent features of CDR3 length could explain the controversial reports about the CDR3 length in SLE (22–26) based on the observation in unsorted B cells or peripheral blood mononuclear cells.

The BCR isotype of SLE also exhibited distinctive features. An over representation of IgA and an increased switching to IgE was reported (15). As IgA plays a major role in immune defense at the mucosal surfaces and the frequently used IGHV in SLE has high affinity for microbial antigens (20), the presence of unknown drivers of the disease in the mucosal microbiome has been suggested.

Rheumatoid arthritis (RA) is an autoimmune disease with systemic manifestations typified by chronic arthritis, mainly in the synovial membrane. Based on the therapeutic efficacy of rituximab in patients with RA and the association of IGHV1-69 polymorphisms with disease susceptibility in RA (27, 28), B cells could play a pivotal role for its pathogenesis. Indeed, increased usage of the IGHV4 family has been observed in RA patients (25, 29, 30). In single-cell BCR repertoire analysis of synovial tissues, clonal expansion of age-associated B cells and memory B cells were

observed (31). In addition, glycosylation of the variable domain of anti-citrullinated protein antibodies was important for its autoreactivity and activity (32), suggesting the importance of post-translational modification as a feature of BCR repertoire.

Systemic sclerosis (SSc) is an idiopathic autoimmune disease characterized by fibrosis of the skin and various organs. B cells are reported to be involved in the pathogenesis of SSc based on altered B cell subset frequency in disease and the efficacy of B cell-targeted therapy (33, 34). The usage of IGHV genes in SSc patients was different from HC and clonotype was more diverse (35). Particularly in SSc-PAH patients, a decrease in the usage of IGHV2-5 and an increase in SHM frequency in expanded clones were observed (36). CDR3 length in peripheral blood mononuclear cells (PBMC) of SSc patients was significantly shorter (35).

Sjögren's syndrome (SS) is an autoimmune disease characterized by reduced exocrine function. In SS patients, peripheral B cell abnormalities, including a predominance of naive B cells and a decrease in memory B cells, have been reported (37). Similar to other autoimmune diseases, skewed IGHV gene usage has been reported (38). While CDR3 length remained unchanged (38), there was an increased ratio of non-synonymous mutations in the CDR region of switched memory B cells and USM Bs (36). In naive B cells, an accumulation of self-reactive clones was observed, suggesting impairment in early B cell tolerance checkpoints (36).

ANCA-associated vasculitis (AAV) is an autoimmune disease characterized by inflammation of small- and medium-sized blood vessels. Given the involvement of anti-neutrophil cytoplasmic antibodies (ANCA) in AAV (39), it is expected that there may be some distinct features in the BCR. However, the repertoire of AAV showed no significant differences in the clonal expansion nor diversification (15). Perhaps the limited number of pathogenic ANCA clones in PBMC may explain the lack of detectable differences (15).

Reports of B cell repertoire analysis of major autoimmune diseases are summarized below (Table 1).

BCR repertoire abnormalities in CD27$^+$IgD$^+$ unswitched memory B cell in autoimmune diseases

The characteristics of BCR repertoire vary by B cell subset, including VDJ gene usage, CDR3 length and isotype frequency (42–45). From our BCR repertoire analysis of sorted B cell subsets, fraction of autoreactive clonotypes in USM B showed a prominent correlation with disease activity in SLE (16), illuminating the disease association of this subset. USM Bs represent a category of innate-like B cells responsible for natural IgM production (46). Natural IgM plays a protective role against autoimmunity by removing apoptotic cells and foreign antigens and suppressing innate inflammation (47, 48).

The origin of USM Bs is still a matter of debate. Some have argued for its correspondence with marginal zone B cells (49, 50), while others have reported the GC-independent origin early in the primary response (51). Still, some argued that they are GC-dependent B cells (50, 52). USM Bs could be further divided into functionally distinctive populations based on IgM positivity (53) or T-bet positivity (54). It remains unclear whether different classes of USM Bs have different origins or if each has multiple origins.

Populational and functional abnormalities of USM B in patients with autoimmune diseases have been reported (55–57). It has been hypothesized that USM B becomes exhausted in the inflammatory milieu, leading to decreased IgM production (55). This is in line with the clinical observation that patients with SLE have low levels of IgM, and that the decreased number of IgM correlates with the duration of disease (58). In RA, USM B was reported to have a proinflammatory feature, decreased capacity of IgM production and altered VDJ gene usage (55). As well, the total number of USM B was decreased but recovered after the effective therapy (55). SSc also showed fewer memory B cell subsets, particularly in USM B, in the peripheral blood (57). In SS, there was a significant decrease in memory B cells, including USM B, in the peripheral blood, while there was a local accumulation of these cells in the salivary glands.

TABLE 1 BCR repertoire features about several autoimmune diseases.

	SLE	RA	SSc	SS
IGHV gene	•Increased usage of IGHV4 gene family (18, 19)	•Association of IGHV1-69 polymorphisms with disease susceptibility (27)	•Biased IGHV gene usage (35) •Reduced IGHV2-5 usage in SSc-PAH (36)	•Altered IGHV gene usage (38)
CDR3 feature	•Shorter or longer CDR3 length (22–26)	•Longer or unchanged CDR3 length (25, 29)	•Significantly shortened CDR3 length (35)	•No significant difference in length (38) •Increased non-synonymous mutations in CDR regions of switched and unswitched memory B cells (40)
Other features	•Increase in IgA clones and class switch from IgA to IgE (15)	•BCR clonotypes in bone marrow and synovium may be protected from depletion (41)	•Increased SHM frequency in expanded clones in SSc-PAH (36)	•Accumulation of self-reactive clones in naïve B cells (40)

This accumulation may possibly be associated with activation or enhancement of local autoimmune responses (56). These reports support the role of USM B in autoimmune diseases.

In recent article (16), we reported that usage of many VDJ genes was skewed in USM B of SLE and other autoimmune diseases compared to healthy controls. We noticed that those differentially used VDJ genes were largely correspondent with the differentially used genes between USM B and naïve B cells in healthy controls. In principal component analysis based on VDJ gene usage, gene usage pattern of USM B in SLE and other diseases were significantly skewed toward the direction into naïve B cells. Gene usage in plasmablasts of SLE was skewed into the same direction as well in the same coordinate plane. For further understanding, we developed a composite score of VDJ gene usage to quantify this skewing. Interestingly, a strong correlation between the skew level of plasmablast gene usage and that of USM B gene usage across patients was observed. These observations implied a shared pathology between these two subsets of SLE.

Also, this gene usage-based score showed significant correlation with the SHM frequency in plasmablast and peripheral helper T cell transcriptome signature, which is associated with GC-independent maturation of B cells (59), in T helper 1 cells. Thus, we proposed that the activation of extrafollicular pathway in SLE could result in the global skew of gene usage of these two subsets.

While much of the research interest has been focused on the extrafollicular maturation of plasmablasts, we have noted that the extrafollicular maturation of USM Bs could also potentially play interesting roles in autoimmune diseases. At steady-state conditions, the IGHV4-34 gene is preferentially expressed in naive B cells. However, it tends to be eliminated in memory B cells through GC. Extrafollicular (GC-independent) pathway is reported to be activated in SLE, bacterial infection as well as COVID-19 infections (21, 60–64). The extrafollicular pathway often bypasses the germinal center maturation process and is known to produce rapid and short-lived antibody responses. The rapid and modestly regulated nature of the extrafollicular pathway could allow for the preferential survival and expansion of IGHV4-34-expressing memory B cells (Figure 1). Additionally, we observed increased *TBX21* expression among presumably extrafollicular pathway driven USM B, suggesting its distinctive function. However, the association between elevated IGHV4-34 usage and other features of USM B in autoimmune diseases, such as decreased population size and decreased IgM production, remains uncertain.
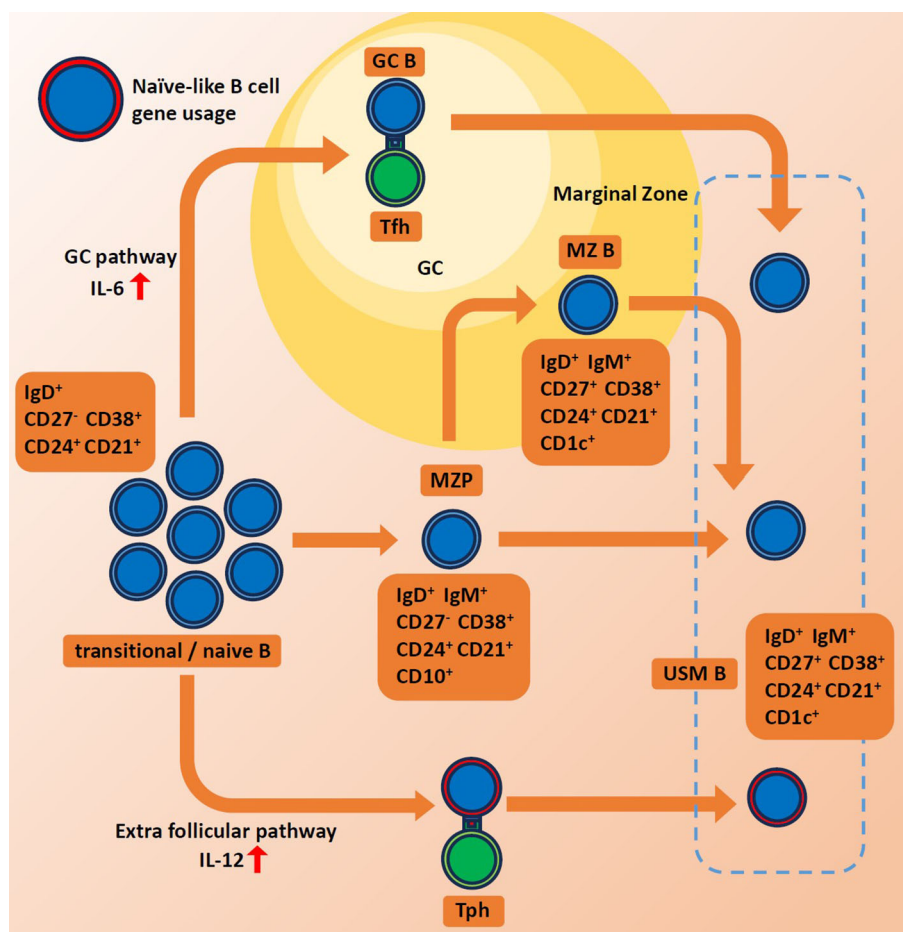

FIGURE 1
Schematics for maturation of unswitched memory B cells. Red arrows indicate high levels of each interferon. GC, germinal center. MZ B, marginal zone B cell. MZP, marginal zone precursor B cell. Tph, peripheral helper T cell. Tfh, follicular helper T cell. USMB, unswitched memory B cell.

Despite the presence of many unknowns, including population heterogeneity, USM Bs have the potential to hold the key to understanding autoimmune diseases.

BCR repertoire as a treatment target and biomarker

B-cell-targeted therapy has already been widely employed in the treatment of autoimmune diseases. Understanding the target of clinically used treatments can guide us toward developing new treatment strategies. For instance, belimumab, a human monoclonal antibody that inhibits B cell stimulating factors, is one of only two biologics approved by the United States Food and Drug Administration for the treatment of SLE. Belimumab treatment has been observed to reduce the fraction of IGHV4-34 in unmutated IgM sequences (65). In line with it, we observed that patients with SLE after treatment with belimumab showed decreased degree of extrafollicular pathway in USM B, which resulted in decreased use of IGHV4-34 (16).

Rituximab (RTX) is the mainstay of B-cell depletion therapy and is widely used to treat autoimmune diseases such as RA, AAV, and idiopathic inflammatory muscle disease (IIM) (66–70). In RA patients, dominant clones before treatment are rapidly cleared from the peripheral blood after treatment, while persistence of clones predicts subsequent non-response (41). In AAV and SLE patients, the majority of clones persistent after RTX treatment was class-switched (15). On the contrary, mycophenolate mofetil (MMF), which belongs to antimetabolites that inhibit purine synthesis in cells, has reduced class-switched clones and increased USM Bs, suggesting the different targets of these drugs on BCR repertoire (15).

Intravenous immunoglobulin (IVIg) acts against activated immune cells and signaling pathways, resulting in anti-inflammatory and immune-modifying effects (71). In patients with IIM, pre-treatment dominant clones disappear with IVIg treatment and the higher the cumulative frequency of pre-treatment dominant clones, the better the response to treatment. It is suggested that treatment responsiveness to IVIg may depend on the composition of the BCR repertoire prior to treatment (72).

Together, different drugs target different aspects of BCR repertoire. Consequently, the BCR repertoire can serve as an indicator of treatment efficacy and a predictor of treatment responsiveness.

Diagnosis of diseases by repertoire analysis is also expected. As a promising example, patients with IgA Nephropathy (IgAN) and healthy individuals could be distinguished based on the repertoire status (73). IgAN is a type of glomerulonephritis, an immune-related disease characterized by renal deposition of IgA (74). BCR repertoire analysis of the B cells of IgAN patients showed shortened CDR3 length. In addition, disease-associated IGH clones were identified, allowing classification of IgAN and healthy individuals. IgAN is generally diagnosed by renal biopsy, which is an invasive test. Less invasive

diagnosis and monitoring of disease status may be possible through repertoire analysis.

Another report has successfully classified patients with celiac disease according to the results of repertoire analysis (75).

Conclusions and perspectives

In this article, we have conducted a review of BCR repertoire analysis of autoimmune diseases, with a specific focus on USM Bs. Within the BCR repertoire of autoimmune diseases, there is a noticeable bias in the usage of VDJ genes. This bias likely reflects underlying B cell biology, such as the activation of extrafollicular pathways. The study of B cell repertoires in autoimmune diseases is still in its early stages. Large-scale repertoire analysis, including single-cell analysis and the incorporation of clinical information, holds the potential to provide further insights and understanding in this field.

USM Bs represent an under-studied population in the context of autoimmunity. Nevertheless, several observations provide support for their potential role in autoimmune diseases. While the precise role of USM Bs remains incompletely understood, it is plausible that a subset of USM Bs, which have matured via the extrafollicular pathway, may contribute to pathogenic processes. Additionally, quantitative and qualitative defects in USM Bs may impair the functionality of protective IgM, potentially leading to disease development.

The results of BCR repertoire analysis have provided various insights into the pathogenesis of autoimmune diseases. However, the BCR repertoire analyses that have been performed so far are mainly conducted on bulk samples. Recently, single-cell repertoire analysis, which can examine BCR information on a single cell basis, has been attracting attention (76). Single-cell repertoire analysis allows pairing of heavy and light chains. For example, a technique for high-throughput mapping of BCR sequences by pairing heavy and light chains has been developed (77, 78). While detailed analysis is undoubtedly crucial, the importance of conducting large-scale cohort studies should not be underestimated, given the heterogeneous nature of autoimmune diseases. Future efforts aimed at a comprehensive analysis of the BCR repertoire in a large cohort will unquestionably enhance our understanding of these conditions.

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