IMMUNOLOGY

Characterizing the BCR repertoire in immune-mediated diseases

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B cells can assume protective or pathogenic roles in immune-mediated diseases (IMDs). Analysis of the B cell receptor (BCR) repertoires in six IMDs provides insights into the diversity of B cell repertoires across diseases as well as into potential pathological mechanisms and the effects of different treatments.

Refers to Bashford-Rogers, R. J. M. et al. Analysis of the B cell receptor repertoire in six immune-mediated diseases. *Nature* **574**, 122–126 (2019)

The diverse range of B cell receptors (BCRs) expressed by the B cells of an individual constitutes their BCR repertoire. Diversification of the naive BCR repertoire is stimulated by exposure of the B cells to antigens and is affected by the context in which antigen exposure occurs. B cell receptor repertoire diversification occurs primarily as a result of two main processes involving immunoglobulin genes: affinity maturation (which occurs through somatic hypermutation (SHM) followed by selection of high-affinity clones)[1] and heavy-chain class-switch recombination (CSR)[2]. These iterative processes select for genetic recombinations that result in B cells that produce antibodies with high affinities and possessing specialized effector functions tailored to the target foreign antigens. Simultaneously, tolerance mechanisms exist that serve as checkpoints to remove or suppress B cells encoding self-reactive BCRs; however, evasion of these checkpoints can give rise to autoimmune conditions. To gain further insight into how B cells contribute to immune-mediated diseases (IMDs), Bashford-Rogers and colleagues have characterized the BCR repertoires in individuals with an IMD[3]. The study's findings reveal intriguing links between the microbiome and B cell-mediated pathogenesis and provide insight into potential strategies for improving current treatment paradigms in autoimmune diseases (FIG. 1).

In this new study[3], the researchers analysed samples from 209 individuals with one of six different IMDs: ANCA-associated vasculitis (AAV), systemic lupus erythematosus (SLE), Crohn's disease, Behçet disease, eosinophilic granulomatosis with polyangiitis (EGPA) and IgA vasculitis (IgAV). To prevent confounding effects from differences in disease duration, activity and treatment, the investigators recruited primarily newly diagnosed, but untreated patients. They used a method to barcode, amplify and sequence the BCR repertoires of the patients using total RNA of sorted B cells. The method produces a barcoded amplicon that encodes the antigen-binding (VDJ) domains and constant regions of the BCR heavy chain. The unique barcodes on each amplicon ensure that the contribution of each B cell to the repertoire is counted only once. Although heavy-chain sequencing excludes the light-chain regions of the BCR, it provides information on the variable region sequence, isotype class, subclass and clonal type frequency.

Bashford-Rogers and colleagues first compared the antibody isotype usage in patients with IMDs and in healthy individuals and discovered that IgA was the dominant isotype in all the patients except those with AAV or EGPA[3]. Furthermore, the over-representation of IgA correlated with increased serum IgA titres, particularly in patients with SLE. Additionally, the IgE isotype was overrepresented in patients with SLE, Crohn's disease or EGPA. By contrast, isotype usage in AAV was similar to that in healthy individuals. The dominance of IgA and IgE isotypes is in line with previous findings that these isotypes are involved in IMD pathology. For instance, IgA–rheumatoid factor immune complexes have been implicated in pathogenesis in the joints of patients with rheumatoid arthritis (RA)[4], and self-reactive IgE antibodies are known to exacerbate inflammatory pathways in SLE[5].

The researchers went on to compare the BCR repertoire diversity with respect to immunoglobulin heavy-chain variable region (IGHV) gene usage[3]. Expression of genes of the IGHV4 family was increased in B cells from patients with SLE, Crohn's disease or EGPA when compared with healthy individuals; notably, BCRs utlilizing IGHV4-34 are known to bind both microbial antigens and autoantigens[6]. Although *IGHV4-34* has previously been associated with SLE, this study[3] extends the association of this gene with other IMDs. Many genes of the IGHV1 family are also associated with certain IMDs and infections[7]. Further, in this new study[3], the gene usage in the different IMDs, with the exception of Crohn's disease, was consistent across both naive and antigen-experienced B cell clonal populations, supporting the idea that a selective expansion of these clones might arise from an early microbial trigger, and not just in response to disease onset.

The complementarity-determining region 3 of the BCR heavy-chain (CDR3) makes up the bulk of the antigen–antibody binding interface. The length and sequence of this region determines antibody specificity, and increased CDR3 lengths are associated with autoimmunity[8]. Bashford-Rogers and colleagues observed that the lengths of CDR3s were increased in the BCRs of IgG-switched and IgA-switched B cells in patients with SLE and in the BCRs of unswitched B cells in patients with Crohn's disease[3]. However, whether the CDR3 regions of BCR heavy chains in patients with the same disease have shared sequence motifs, as has been shown in other established autoimmune indications such as RA, remains unknown.

BCR repertoire size and diversity is a direct result of B cell clonal expansion, CSR and SHM. Clonal expansion and diversification across different isotypes was increased in patients with Crohn's disease, SLE or EGPA, decreased in patients with Behçet disease and unchanged in patients with AAV or IgAV when compared with healthy individuals[3]. Interestingly, levels of SHM did not

vary across the IMDs. The observation that the BCR repertoire in patients with AAV or IgAV do not vary from that of healthy individuals is inconsistent with the established role of autoantibodies in these diseases. In these diseases, it could be informative to further define whether B cell activation states or dysregulated transcriptional programs promote disease.

Interestingly, the researchers investigated the effects of two well-known therapies on the BCR repertoire of patients with SLE or AAV[3]. The two treatment regimens — rituximab (RTX), which depletes CD20-positive B cells, or treatment with mycophenolate mofetil (MMF), which inhibits cell proliferation — had widely differing effects on the BCR repertoire. After RTX and consequent B cell depletion, the persisting cells were predominantly clonally expanded and class-switched, and could thereby contribute to post-treatment relapse. By contrast, MMF treatment resulted in reduced class switching and clonality, with concomitant increases in IgM-positive B cells and IgD-positive B cells. However, what gene expression changes in the B cells contribute to therapeutic outcomes is unknown and could be the subject of future investigations. Nevertheless, in a treatment paradigm similar to that proposed for the use of RTX followed by belimumab (which blocks B cell-activating factor (BAFF)) to maintain remission[9], the results from this study[3] have implications for the potential use of RTX followed by MMF to promote therapeutic efficacy in both SLE and AAV (FIG. 1).

One of the limitations of repertoire analysis with BCR heavy-chain sequencing is that no paired light-chain information is provided. The BCR light chain pairs with the heavy chain to form the antibody binding site, and hence the light chain directly contributes to the binding site and thereby the specificity and binding properties of an antibody. Paired heavy-chain and light-chain sequences, particularly for expanded or persistent post-treatment B cell clones, are required for more comprehensive analysis of the antibody repertoire as well as for the determination of antigen specificity through recombinant antibody expression[10]. In addition to characterizing the functional antibody repertoire in IMDs, further studies to investigate the cross-reactivity of expanded clones to commensal bacteria are needed to identify potential microbial triggers.

The development and accessibility of large-scale sequencing is transforming our understanding of human disease. Bashford-Rogers and colleagues applied large-scale sequencing to study features of the B cell repertoires associated with IMDs, revealing marked differences in variable gene and isotype usage that provide important insights into these diseases[3]. Application of this approach and related approaches to additional IMDs is anticipated to considerably advance our understanding of the underlying disease mechanisms and lead to the development of next-generation therapeutic approaches for a variety of IMDs.

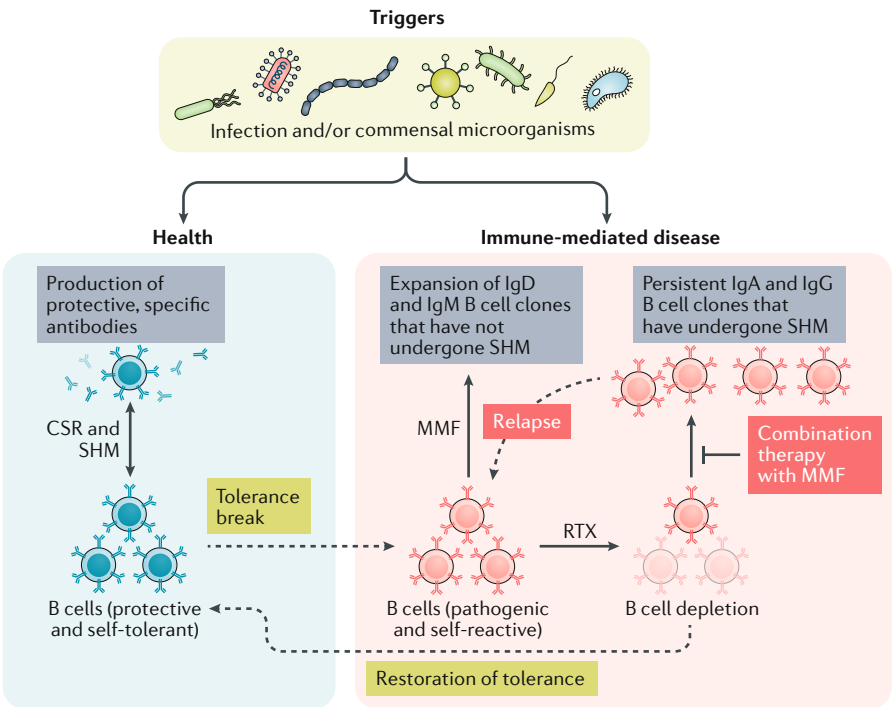

Fig. 1 | **Model for the role of B cells in immune-mediated diseases.** Healthy individuals possess B cell receptor (BCR) repertoires that are largely tolerant to self-antigens. Upon antigen exposure to infectious microbes or in response to commensal organisms, BCRs diversify by somatic hypermutation (SHM) and class-switch recombination (CSR). Production of protective, specific antibodies contributes to the prevention and/or resolution of microbial infections. Tolerance breaks can lead to the expansion of autoreactive pathogenic B cells, promoting disease. B cell depletion therapies such as rituximab (RTX) can reduce development of pathogenic B cells, promoting restoration of tolerance. However, a small subset of persistent, class-switched, somatically hypermutated clones might continue to expand, promoting relapse. Mycophenolate mofetil (MMF) inhibits the proliferation of somatically hypermutated and class-switched B cells but does not deplete them. Combination therapy (for example, with MMF and RTX) might promote more effective therapeutic outcomes.

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Competing interests
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