Mechanisms of Action and Resistance of B Cell-Targeting Monoclonal Antibodies in Autoimmune Diseases

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Abstract. B lymphocytes are the core of the pathogenesis of various autoimmune diseases, playing an irreplaceable role in the disease process as plasma cell precursors that produce autoantibodies, potent antigen-presenting cells, and key regulators of immune responses through cytokines. Therefore, therapies targeting B cells have become an important cornerstone for treating such diseases, and exploring their related mechanisms and optimizing treatment plans has significant clinical significance. This article focuses on B-cell targeted monoclonal antibodies (mAbs), systematically explaining their mechanisms of action and drug resistance issues. Regarding drug resistance, the article analyzed its multifactorial mechanisms, including target-related changes (such as CD20 mutations or downregulation), host factors (such as Fc γ R polymorphism), and the influence of tumor microenvironment and compensatory survival pathways. This article underscores the transformative impact of advanced techniques like single-cell multi-omics and CRISPR screens in biological inquiry and elucidates approaches to overcome resistance, including developing next-generation antibodies (bispecifics, ADCs), rational combo therapies, and personalized medicine. This article systematically reviews the mechanism of action and drug resistance issues of B-cell targeted monoclonal antibodies, which not only deepens the understanding of related biological processes but also provides theoretical references for optimizing treatment plans and avoiding drug resistance in clinical practice, helping to promote the precise and efficient development of autoimmune disease treatment.

Keywords: B-cell-targeting monoclonal antibody; autoimmune diseases; mechanism of action; resistance.

1. Introduction

Autoimmune diseases represent a spectrum of more than 100 chronic conditions, all originating from a breakdown in self-tolerance that misdirects the immune system against the body's own tissues. In disorders like rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and multiple sclerosis (MS), the pathogenesis involves a complex interplay among immune cells, wherein B lymphocytes occupy a pivotal role. Their contribution to autoimmunity is threefold: differentiation into plasma cells that secrete autoantibodies, presentation of autoantigens to T cells, and secretion of pro-inflammatory cytokines [1]. This multifaceted role makes B cells an exceptionally attractive therapeutic target [2].

The introduction of monoclonal antibody (mAb) technology has transformed the treatment paradigms in oncology and autoimmunity. mAbs achieve unparalleled specificity by precisely binding to cell surface molecules, a feat not possible with conventional immunosuppressants. A landmark advancement in this domain was the repurposing of rituximab—a chimeric anti-CD20 mAb first approved for B-cell lymphomas—for autoimmune conditions, including RA and ANCA-associated vasculitis [3]. The CD20 antigen is an ideal target due to its expression on pre-B, mature, and memory B cells, and its absence on hematopoietic stem cells, pro-B cells, and plasma cells. This expression profile enables the profound depletion of circulating B cells while sparing the capacity for immune reconstitution and protecting the antibody output from long-lived plasma cells. Rituximab's success catalyzed the creation of enhanced anti-CD20 agents like ofatumumab and obinutuzumab, which were designed for superior effector functions [4].

Complementing direct depletion strategies, an alternative approach focuses on disrupting the survival signals essential for B-cell homeostasis. B-cell activating factor (BAFF), a cytokine critical

for B-cell survival and maturation, is often present at pathologically elevated levels in autoimmune diseases like SLE. This surplus promotes the survival and proliferation of autoreactive B cells. Belimumab, a human mAb that binds and neutralizes soluble BAFF, embodies this strategy. Instead of inducing widespread B-cell death, it aims to restore immune homeostasis by selectively starving autoreactive B cells of essential survival signals, thereby reducing the production of autoantibodies [5].

Despite the transformative impact of these therapies, a substantial portion of patients either fail to respond adequately (primary resistance) or lose their response over time (secondary or acquired resistance). This clinical challenge underscores the complexity of B-cell biology and the intricate interplay between the therapeutic agent, the host immune system, and the disease microenvironment. Resistance can arise from a multitude of mechanisms, ranging from alterations in the drug target itself, such as mutations or downregulation of the CD20 antigen, to host-related factors, like genetic polymorphisms in Fc receptors that mediate antibody effector functions, or the development of neutralizing anti-drug antibodies.

Understanding the exact molecular and cellular mechanisms behind the mechanisms of action and resistance of these potent drugs is crucial for optimizing their applications. This article details the established and emerging action mechanisms for major anti-CD20 (rituximab, ofatumumab, obinutuzumab) and anti-BAFF (belimumab) monoclonal antibodies, and explores in depth the multifaceted mechanisms of therapeutic resistance. The focus is on cutting-edge research methods such as single-cell multi-omics and CRISPR-based screening, which provide unprecedented insights into analyzing these complex biological processes.

2. B Cell-Targeting Monoclonal Antibodies: Action Mechanisms

The clinical benefits of B-cell-targeting monoclonal antibodies in autoimmune disorders are primarily mediated by their mechanisms of action, which, while distinct, frequently exhibit overlap. These mechanisms principally fall into two categories: the direct elimination of B cells through cytotoxic effects and the modulation of B cell survival and activation pathways. Anti-CD20 mAbs are the prototypic B-cell depleting agents. Their primary goal is to eliminate CD20-positive B cells from circulation and tissues, thereby disrupting the autoimmune cascade. The mechanisms of cytotoxicity depend on the specific antibody's structure, particularly its Fc region and its binding epitope on the CD20 molecule (See Fig. 1).

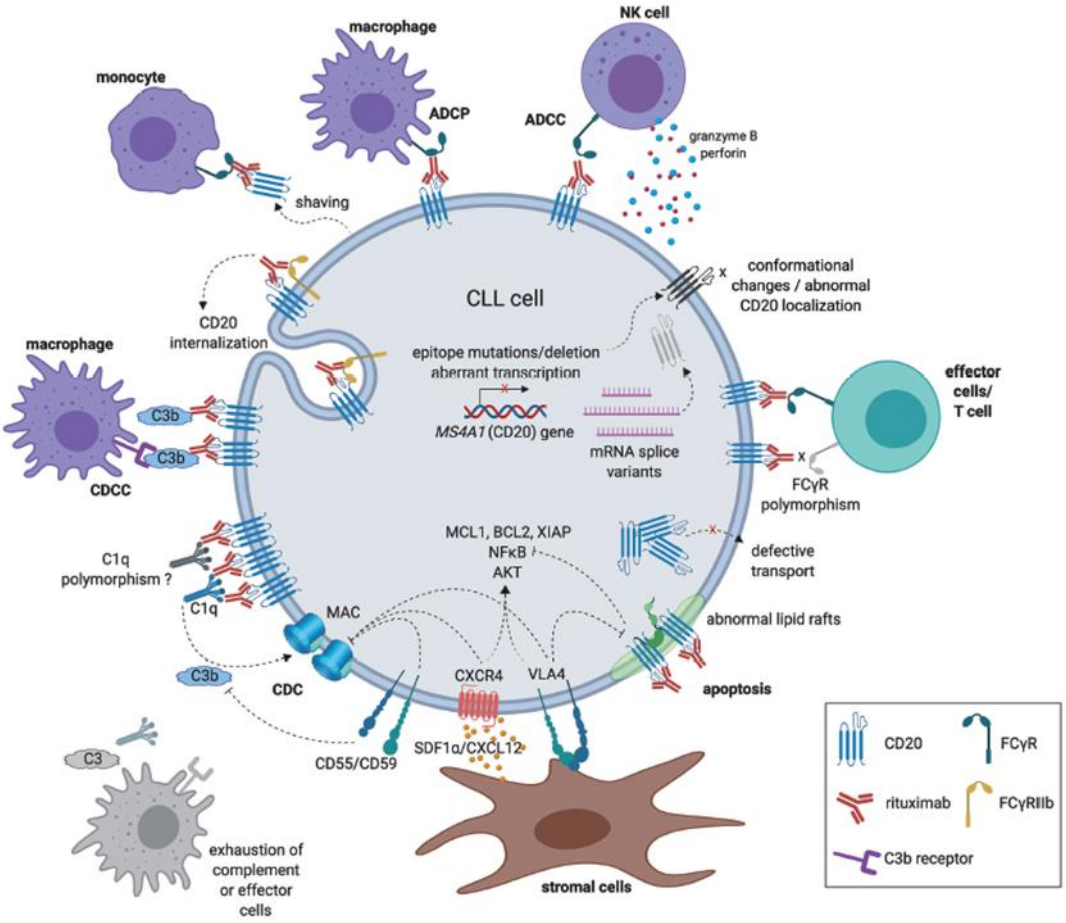

Fig. 1 Major action mechanism of anti-CD20 monoclonal antibodies [6]

Rituximab, a chimeric mouse-human IgG1 mAb, has been the workhorse of B-cell depletion for over two decades. As a Type I antibody, it effectively partitions into lipid rafts on the B-cell membrane upon binding to CD20. Its efficacy is attributed to a combination of three major mechanisms [6]. Firstly, antibody-dependent cellular cytotoxicity (ADCC) is regarded as the predominant pathway. In this process, the Fc portion of the bound rituximab is engaged by Fcγ receptors (notably FcγRIIIA/CD16a) expressed on effector immune cells—primarily natural killer (NK) cells and macrophages. This engagement triggers the effector cell to release cytotoxic granules containing perforin and granzymes, inducing apoptosis in the targeted B cell. The second is complement-dependent cytotoxicity (CDC). When rituximab binds to multiple CD20 molecules in close proximity within a lipid raft, its Fc segment can bind to the first component of the classical complement pathway, C1q, initiating a series of enzymatic reactions and ultimately forming a membrane attack complex (MAC) on the surface of B cells. The MAC creates pores in the cell membrane, leading to osmotic lysis and cell death. The third is direct apoptosis, and rituximab can transduce pro-apoptotic signals directly upon cross-linking of CD20 molecules, leading to programmed cell death independent of immune effector cells or complement. However, the relative contribution of this pathway in vivo is considered subsidiary to ADCC and CDC.

Ofatumumab is a fully human IgG1 mAb developed to improve upon rituximab. Although also a type I antibody, it recognizes a distinct, membrane-proximal epitope on CD20. This distinct binding site allows for more efficient recruitment and activation of C1q. Consequently, ofatumumab exhibits significantly more potent CDC activity than rituximab. While it also mediates ADCC, its primary advantage lies in its enhanced ability to harness the complement system for B-cell lysis. Its fully human nature also reduces the risk of immunogenicity compared to the chimeric rituximab [7].

Obinutuzumab (GA101) represents a significant advancement in antibody engineering and is a humanized, sugar-engineered type II IgG1 monoclonal antibody. Its unique characteristics give it a different and superior mechanism of action compared to type I antibodies. The key innovation in

obinutuzumab is the afucosylation of the N-glycans in its Fc region. This modification dramatically increases its binding affinity for the activating FcγRIIIA receptor on NK cells and other effector cells. The result is substantially more potent ADCC induction, leading to more efficient B-cell killing even at lower antibody concentrations or in the presence of competing IgG. Unlike Type I antibodies, obinutuzumab does not efficiently translocate into lipid rafts and is less effective at inducing CDC. Instead, they induce a much stronger form of homotypic aggregation and non-apoptotic programmed cell death. This direct cell-killing mechanism is significantly more potent than the apoptosis induced by rituximab. Due to its binding orientation and inability to form the necessary hexameric structures, obinutuzumab is a poor inducer of CDC. Its therapeutic effect relies almost entirely on superior ADCC and direct cell death.

Belimumab works differently from anti-CD20 drugs in that it does not directly kill B cells, but targets the cytokine B cell activating factor (BAFF), which is crucial for B cell survival and maturation. In autoimmune diseases such as systemic lupus erythematosus, BAFF levels often increase in a pathological manner, creating a "survival-promoting" environment that allows self-reactive B cells to bypass normal tolerance checkpoints, proliferate, and differentiate into plasma cells that produce autoantibodies(See table 1). Belimumab is a fully human IgG1λ monoclonal antibody that binds soluble BAFF with high affinity, inhibiting its engagement with BAFF receptors (BAFF-R, TACI, and BCMA) on B cells. By sequestering BAFF, the antibody effectively withdraws essential survival signals, preferentially triggering apoptosis among autoreactive B-cell clones. Clinically, this leads to a progressive reduction in circulating transitional, naïve, and activated B cells, as well as short-lived plasma cells. Over time, belimumab promotes the normalization of the B-cell compartment, which correlates with diminished autoimmune activity, lower autoantibody titers, and improved clinical outcomes [8]. Notably, owing to their BAFF-independent persistence, memory B cells and long-lived plasma cells are largely unaffected by this treatment.

Table 1. Selection of mAbs being approved or tested for autoimmune diseases [6-8]

Drug	Action Mechanism	Therapeutic Indications in Autoimmune Diseases
Rituximab	Chimeric anti-CD20 mAb	Approved: Rheumatoid arthritis (RA); Granulomatosis with polyangiitis and microscopic polyangiitis
Ofatumumab	Fully humanized anti-CD20 mAb	Approved: Relapsing multiple sclerosis (RMS)
Obinutuzumab	Fully humanized anti-CD20 mAb	Phase II complete: Systemic lupus erythematosus (SLE) Lupus Nephritis (LN)
Belimumab	Anti-BAFF mAb	Approved: Systemic lupus erythematosus (SLE) Lupus nephritis (LN)

3. Mechanisms of Resistance

The resistance of B-cell targeted therapy is divided into primary (initial non-response) and secondary (late-stage non-response), with mechanisms involving target antigens, host factors, and tissue microenvironment. The alteration of the CD20 antigen in the target-related mechanism is crucial. Autoreactive B cells may lose or downregulate CD20 expression to evade recognition. MS4A1 gene mutations (such as extracellular loop or C-terminal deletion) can reduce antibody binding. CD20 antibody complexes can also be removed through internalization or macrophage "scraping", reducing surface antigen density, and type I antibodies are more susceptible to this effect[6,9,10]. Host factors also limit efficacy, with FCGR3A gene polymorphism leading to differences in Fc γ RIIIA affinity. Patients with low-affinity genotypes have a poor response to

rituximab, while sugar-engineered antibodies are less affected. Complement system defects or B-cell expression of CD55/CD59 can weaken the CDC effect, and the host may also produce anti-drug antibodies (especially chimeric antibodies) against therapeutic antibodies, interfering with drug action or clearance. The organizational microenvironment promotes drug resistance through various means. B cells in lymph nodes or inflamed synovium can receive signals such as CD40L and IL-4 from follicular dendritic cells, activating survival-promoting pathways such as NF-κB. Target cells may reduce their dependence on BAFF and survive through compensatory signals such as APRIL. Impaired NK cell toxicity in inflamed tissues can also weaken ADCC efficiency.

4. Research Methods for Studying Mechanism and Resistance

The mechanism of action and drug resistance of monoclonal antibodies are complex, thus requiring the use of complex research tools that can provide high-resolution data at both single-cell and functional gene network levels. This field is no longer limited to bulk analysis, but has shifted towards techniques for analyzing cellular heterogeneity and causal genetic relationships. Single-cell multi-omics technology has revolutionary significance, as it can help researchers break down seemingly uniform B-cell populations into functionally distinct subgroups, and thus understand their individual responses to treatment [11,12]. As a cornerstone technology, single-cell RNA sequencing (scRNA-seq) profiles the transcriptomes of thousands of individual cells, uncovering rare B-cell subsets that may possess intrinsic resistance mechanisms. This approach also permits longitudinal tracking of transcriptional dynamics throughout treatment, revealing activation of resistance pathways—for instance, identifying subpopulations that upregulate anti-apoptotic genes or downregulate MS4A1 (encoding CD20) in response to rituximab. An important extension of scRNA-seq, CITE-seq (Cellular Indexing of Transcriptomes and Epitopes by Sequencing), simultaneously quantifies RNA and surface protein expression within the same cell using antibody–oligonucleotide conjugates. This is very valuable for studying monoclonal antibody resistance, as it can directly correlate cell surface CD20 protein levels with their specific gene expression profiles and identify other surface markers associated with drug resistance.

Functional genomics based on CRISPR is also an authoritative tool for identifying genes that regulate drug sensitivity and resistance. Whole genome CRISPR screening is widely used: researchers introduce a library of guide RNAs (gRNAs) targeting each gene in the genome into a B cell population, and then treat the cells with monoclonal antibodies such as rituximab. Surviving cells are enriched with two types of gRNAs: one is gRNAs that knock out essential genes for drug efficacy (sensitive genes), and the other is gRNAs that knock out negative regulatory factors for drug resistance pathways (resistance genes). By sequencing gRNAs in the surviving population, a complete list of genes that regulate drug response can be obtained. Perturb seq/CROP seq (CRISPR omics) is a more advanced method that combines pooled CRISPR screening with scRNA seq readings[13]. Each cell receives specific gene perturbations (such as gene knockout), and then its entire transcriptome is sequenced. This allows researchers to not only understand whether gene knockout leads to drug resistance, but also understand the mechanism of drug resistance by observing the detailed transcription results of the knockout. For example, it has been found that knocking out specific E3 ubiquitin ligases can prevent CD20 downregulation and alter multiple survival pathways.

Advanced in vitro and in vivo models are also crucial for validating high-throughput screening findings and studying drug resistance in environments closer to physiology. Patient-derived cultures and organoids are important in vitro models, and the use of primary B cells isolated from patients who respond or do not respond to treatment can help directly investigate clinical drug resistance mechanisms. Co-culture systems containing stromal cells or T cells can also partially simulate protective tissue microenvironments. The humanized mouse model provides an in vivo research platform. By transplanting the human immune system (such as hematopoietic stem cells), the efficacy and drug resistance development of human-specific monoclonal antibodies can be studied. It can track the clearance of human B cells in different tissues and isolate drug-resistant B cells for further analysis.

Although not emphasized in the specific results of this topic, the use of in vivo imaging techniques (such as fluorescently labeled antibodies or B cells) in these models can provide real-time spatial and temporal information, helping to understand the dynamic processes of drug distribution, target binding, and cell killing and resistance in live animals.

5. Strategies to Overcome Resistance

There are various optimization strategies available for drug resistance mechanisms. The next generation of antibodies can enhance their efficacy through modification. Opinizumab is sugar-engineered to increase Fc γ RIIIA affinity, bypassing host drug resistance. Opinizumab targets different CD20 epitopes to enhance CDC. In the future, antibodies can target more stable epitopes to reduce antigen loss. Combination therapy works synergistically through multiple pathways. The combination of anti-CD20 antibodies and PI3K/BTK inhibitors can block the compensatory survival pathway of B cells. Drugs such as IL-15 activate NK cells to enhance ADCC. The combination of anti-CD20 scavengers and belimumab can kill existing B cells and cut off the survival signal of new cells, improving the durability of the therapeutic effect. The novel antibody construct provides a new mechanism, where bispecific antibodies can simultaneously target B cell CD20 and T cell CD3, or CD20 and CD19, avoiding antigen loss and escape. Antibody drug conjugates (ADCs) deliver toxins directly to kill cells through antibody delivery, without relying on ADCC/DCs, and remain effective against drug-resistant cells. Personalized medicine achieves precise treatment by screening biomarkers (such as Fc γ R genotype and CD20 expression) before treatment to guide medication selection. During treatment, MS4A1 mutations or anti-drug antibodies are monitored through liquid biopsy to adjust the plan in a timely manner to avoid recurrence.

6. Conclusion

Monoclonal antibodies targeting B cells represent a cornerstone therapeutic strategy for autoimmune diseases. The evolution from first-generation rituximab to second-generation ofatumumab and third-generation obinutuzumab illustrates a continuous effort to develop agents with improved efficacy and specificity. These therapeutics refine therapeutic outcomes through engineered enhancements in mechanisms such as complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC). This review has delineated the principal modes of action of these agents, encompassing B-cell depletion via anti-CD20 antibodies and the modulation of B-cell survival through BAFF inhibition by belimumab. Furthermore, it highlights that treatment resistance is driven by a range of adaptive mechanisms, including antigen-related alterations (e.g., CD20 downregulation or mutation), host-specific factors (e.g., Fcγ receptor polymorphisms and anti-drug antibodies), and microenvironmental cues (e.g., pro-survival signaling and compensatory pathways). By elucidating the mechanistic basis of both therapeutic action and resistance, this review provides a conceptual foundation for refining clinical strategies, guiding novel drug development, and advancing personalized treatment regimens to improve precision medicine. The current limitation is that the network of drug-resistant molecules has not been fully elucidated, and therapies are inadequate in responding to complex drug-resistant situations. In the future, technologies such as single-cell multi omics and CRISPR will further elucidate the mechanisms of drug resistance. The combination therapy of novel drugs such as bispecific antibodies and ADCs with personalized treatment guided by biomarkers is expected to break through the bottleneck of drug resistance, promote more efficient and durable B-cell targeted therapy, and benefit more patients.

References

[1] Zhang Z, Xu Q, Huang L. B cell depletion therapies in autoimmune diseases: Monoclonal antibodies or chimeric antigen receptorbased therapy? Frontiers in Immunology. 2023; 14:1126421.

[2] Stathopoulos P, Dalakas MC. Evolution of Anti-B Cell Therapeutics in Autoimmune Neurological Diseases. Neurotherapeutics. 2022; 19(3): 691-710.

[3] Dalakas MC. B cells as therapeutic targets in autoimmune neurological disorders. Nature Clinical Practice Neurology. 2008; 4(10): 557-567.

[4] Hauser SL, Bar-Or A, Cohen JA, et al. Ofatumumab versus teriflunomide in multiple sclerosis. New England Journal of Medicine. 2020; 383(6): 546-557.

[5] Goldberg A, Katzap E. Belimumab for the treatment of systemic lupus erythematosus. Int. J. Clin. Rheumatol, 2010; 5(4): 407-413.

[6] Pavlasova G, Mraz M. The regulation and function of CD20: an “enigma” of B-cell biology and targeted therapy. Haematologica. 2020; 105(6): 1494-1506.

[7] Kubota T, Niwa R, Satoh M, et al. Engineered therapeutic antibodies with improved effector functions. Cancer Science. 2009; 100(9), 1566-1572.

[8] Blair HA, Duggan ST. Belimumab: A Review in Systemic Lupus Erythematosus. Drugs. 2018; 78(3): 355-366.

[9] Arp AB, Elfrink S, Spriel AB. Improving therapeutic CD20 antibodies requires insight into their mechanism of action. Crit Rev Oncog. 2020; 25(3): 251-273.

[10] Torka P, Barth M, Ferdman R, et al. Mechanisms of resistance to monoclonal antibodies (mAbs) in lymphoid malignancies. Curr Hematol Malig Rep. 2019; 14(5): 426-438.

[11] Papalexi E, Satija R. Single-cell RNA sequencing to explore immune cell heterogeneity. Nature Reviews Immunology. 2018; 18(1): 35-45.

[12] Nicholas CA, Smith MJ. Application of single-cell RNA sequencing methods to develop B cell targeted treatments for autoimmunity. Frontiers in Immunology. 2023; 14:1103690.

[13] Azeez SS, Hamad RS, Hamad BK, et al. Advances in CRISPR-Cas technology and its applications: revolutionising precision medicine. Frontiers in Genome Editong. 2024; 6:1509924.