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Biotechnological approaches to exploring autoimmune pathways in Neurological diseases: Current progress and future prospects

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ABSTRACT

Neurological diseases that are among the most debilitating long-term afflictions affecting the nervous system include multiple sclerosis (MS), myasthenia gravis (MG), neuromyelitis optical spectrum disorder (NMOSD), and autoimmune encephalitis (AIE). Despite significant improvements in conventional immunosuppressive therapies, a significant portion of patients continue to experience severe long-term neurological and behavioral side effects and do not respond to treatment. Over the past 10 years, there has been a significant shift in the discovery and therapeutic targeting of autoimmune pathways due to biotechnological developments. Single-cell RNA sequencing (scRNA-seq), CRISPR functional genomics screens, spatial transcriptomics, phage display technologies, and artificial intelligence (AI)-driven analysis has significantly changed researchers' comprehension of the cellular and molecular mechanisms underlying these disorders. These research platforms are supported by innovative treatment strategies, including.CAR-T cell therapy, bispecific antibodies, next-generation checkpoint modulators, and tailored cytokine therapies that target pathogenic immune pathways. The state of biotechnological breakthroughs in autoimmune neurology is summarized in this comprehensive review, which looks at both therapeutic and discovery methods, highlights significant challenges, and outlines prospective future improvements that might significantly improve patient outcomes.

KEY WORDS

Autoimmune neurological diseases; Single-cell omics; CRISPR screens; CAR-T therapy; Spatial transcriptomics; Precision medicine; Multiple sclerosis; Myasthenia gravis

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Introduction

Clinical burden and unmet needs

Dysregulated immune assaults on the central or peripheral nervous system are the hallmark of a diverse range of illnesses known as autoimmune neurological disorders [1]. Approximately 2.9 million people worldwide suffer from multiple sclerosis, with yearly incidence rates varying by region from 1 to 10 per 100,000 [2,3]. The most prevalent autoimmune neuromuscular junction condition, myasthenia gravis, affects 15–20 people out of every 100,000 in affluent countries [4,5]. Despite being less common (1–5 per 100,000), neuromyelitis optica spectrum disease (NMOSD) has much greater morbidity and recurrence rates than multiple sclerosis [6]. A major cause of acquired peripheral neuropathy, chronic inflammatory demyelinating polyneuropathy (CIDP) affects 0.5–2 out of every100,000 people worldwide [7]. Even with the availability of immunosuppressive drugs and disease-modifying therapies (DMTs), a sizable percentage of patients are still intolerant or resistant to first-line therapy. About 30–40% of MS patients on interferon-beta therapy have insufficient disease control, which calls for treatment escalation [3]. Only 50–70% of individuals with myasthenia gravis reach mild manifestation status with current treatments, and long-term steroid reliance is still an issue [8]. Additionally, there is mounting evidence of the severe long-term cognitive, psychological, and physical consequences linked to autoimmune encephalitis, with up to 50% of survivors reporting chronic cognitive impairment two years after diagnosis (Figure 1) [9].

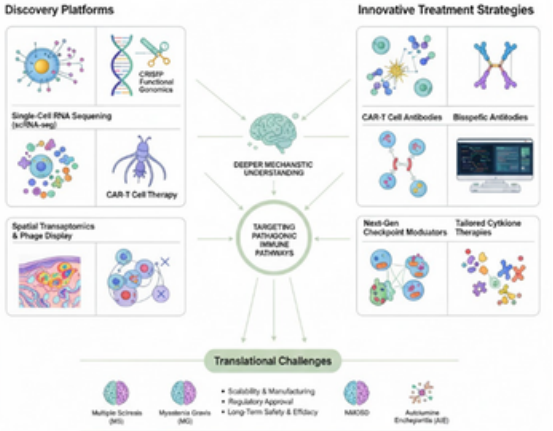

Figure 1. Autoimmune neurology: Discovery and therapeutics targets.

Paradigm shift: From broad immunosuppression to precision targeting

In the past, broad-spectrum immunosuppressive drugs were used to treat autoimmune neurological disorders. These drugs

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indiscriminately reduced immune function, which led to poor disease management, serious toxicity, infections, and cancer [10,11]. A significant paradigm shifts toward comprehending and precisely addressing the cellular and molecular pathways behind autoimmunity has occurred during the last ten years [12]. Three major advancements have come together to facilitate this shift: (1) cutting-edge biotechnological platforms that allow single-cell and functional genomic analysis [13,14], (2) the identification of new therapeutic mechanisms functioning at the nexus of cell biology and immunotherapy [15,16], and (3) evidence showing that "immune reset" through cell therapy can induce sustained drug-free remission in previously refractory cases [17,18]. The current study summarizes the state-of-the-art biotechnological methods for comprehending autoimmune neurological circuits, including therapeutic applications and discovery tools. It highlights recent successes and suggests future possibilities that might improve patient outcomes.

Biotechnological Tools for Exploring Autoimmune Pathways

Single-Cell RNA sequencing: Mapping cellular heterogeneity and rare pathogenic subsets

Considering its unparalleled resolution of cellular composition, states, and functional variety, single-cell RNA sequencing (scRNA-seq) has become a fundamental method for analyzing immunological heterogeneity in autoimmune neurological disorders [19]. By capturing gene expression profiles at the level of individual cells, scRNA-seq allows for the objective identification of distinct immune cell populations and disease-specific transcriptional programs, in contrast to bulk RNA sequencing, which averages transcriptomic signals across heterogeneous cell populations and obscures rare but pathogenic subsets [20,21]. By identifying previously unknown subsets of autoreactive T cells, B cells, monocytes, microglia, and innate lymphoid cells, scRNA-seq has revolutionized our knowledge of pathogenic immune processes in autoimmune illnesses of the central and peripheral nervous systems [22]. For instance, clonally increased cytotoxic $CD8^+$ T cells, pathogenic Th17-like $CD4^+$ T cells, and pro-inflammatory microglial states that correlate with disease activity and neurodegeneration have been discovered by scRNA-seq investigations in MS lesions and cerebrospinal fluid [23,24]. Similarly, scRNA-seq has identified separate B-cell and plasmablast populations enriched for interferon-responsive and antibody-secreting gene signatures in neuromyelitis optica spectrum disease (NMOSD), offering mechanistic insights into antibody-mediated astrocytopathy [25]. The capacity of scRNA-seq to capture dynamic immunological states and cellular plasticity rather than rigid cell types is a key benefit. Reconstructing immune differentiation pathways is made possible by trajectory and pseudo time studies, which show how naïve or regulatory immune cells change into pathogenic effector phenotypes in inflammatory settings [26]. Finding fatigue, activation, and tissue-resident memory states that affect the chronicity of a disease and how well a therapy works has been very helpful [27]. The tracking of clonal growth and antigen specificity in autoimmune neuroinflammation is further made possible by the integration of scRNA-seq withcomplementary modalities like single-cell T-cell receptor (TCR) and B-cell receptor (BCR) sequencing [28]. The idea of compartmentalized immunity that is resistant to traditional systemic immunosuppression has been supported by these methods, which have shown that persistent clonal immune populations may exist inside the central nervous system [29]. ScRNA-seq has important translational implications that go beyond mechanistic discovery. The application of disease-associated gene signatures found at single-cell resolution for patient stratification, treatment response prediction, and the discovery of new pharmacological targets is growing [30]. Furthermore, precision immunotherapies that selectively deplete or reprogram pathogenic immune subsets while maintaining protective immunity have been developed thanks to scRNA-seq data, which is a crucial step toward individualized treatment plans for autoimmune neurological disorders [31].

Technical workflow and advances

In order to ensure accurate cell-of-origin assignment, most modern single-cell RNA sequencing (scRNA-seq) platforms depend on high-throughput microfluidic technologies that enable individual cells to be physically segregated into separate reaction compartments [32]. In droplet-based systems, such as those that employ nanoliter-scale emulsions, single cells are encapsulated with barcoded gel beads coated with oligonucleotides containing cell-specific barcodes and unique molecular identifiers (UMIs) [33]. Following cell lysis, polyadenylated messenger RNA molecules hybridize to these barcoded primers within droplets, preserving transcript identity and cellular provenance during further processing [34]. After mRNA is captured, reverse transcription generates complementary DNA (cDNA), which is subsequently amplified to yield sufficient material for sequencing [35]. Libraries with thousands to millions of individual cells are then subjected to next-generation sequencing. This typically yields a sequencing depth of around 50,000–100,000 reads per cell, allowing for the accurate identification of both highly expressed and low-abundance transcripts associated with pathogenic signaling and immune activation [36]. Advances in chemistry and sequencing methods have led to considerable increases in sensitivity, transcript coverage, and throughput, enabling comprehensive analysis of complex immune landscapes in autoimmune neurological tissues [37]. Computational analysis is a crucial step in the scRNA-seq process. The initial quality control processes exclude low-quality cells, doublets (situations when many cells are trapped inside a single droplet), and dying or stressed cells with aberrant mitochondrial gene expression [38]. Data normalization techniques take into consideration technical variability arising from differences in capture efficiency and sequencing depth when visualizing high-dimensional transcriptomic data. Dimensionality reduction methods such as t-distributed stochastic neighbor embedding (t-SNE) and uniform manifold approximation and projection (UMAP) come next [39]. Unsupervised clustering techniques are then used to identify transcriptionally distinct cell types and functional states without any prior assumptions [40]. One kind of downstream analysis used to identify possible treatment targets, transcriptional regulators, and disease-associated pathways is differential gene expression testing between clusters [41]. Trajectory and pseudo time investigations, which also demonstrate dynamic transitions from naïve or regulatory immune states to pathogenic effector phenotypes, enable the reconstruction of cellular differentiation and activation pathways [42]. Recent technological advancements have made it possible for scRNA-seq to move beyond transcriptomics. The integration of RNA sequencing with simultaneous surface protein expression measurement using CITE-seq (cellular indexing of transcriptomes and epitopes by sequencing), which

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connects transcriptional and proteomic information within the same cell, has enabled multimodal single-cell profiling [43]. In autoimmune neurology, where pathogenic and non-pathogenic immune subsets may be identified by subtle differences in receptor expression or activation markers, this technique has been particularly useful [44]. Together, these technical developments have made scRNA-seq a powerful, scalable, and therapeutically valuable tool for comprehending immune dysregulation in autoimmune neurological diseases.

Applications in autoimmune neurology

Single-cell RNA sequencing has provided important insights into autoimmune neurological diseases by identifying harmful immune subsets that were hard to distinguish using bulk techniques. ScRNA-seq analysis of thymic tissue and peripheral blood showed a unique CD180-negative B-cell group in myasthenia gravis (MG) patients. These patients had high anti-acetylcholine receptor (AChR) antibody levels and severe disease symptoms [45,46]. The gene activity of these cells shows increased expression of genes involved in immunoglobulin class-switch recombination, germinal center reactions, and T-cell-dependent B-cell activation. This explains why high-affinity autoreactive plasma cells persist, even with standard immunosuppressive treatments [47]. The discovery of this harmful B-cell population supports treatment strategies that target both B cells and T-cell help, rather than focusing solely on removing antibodies. ScRNA-seq studies of brain lesions, peripheral blood, and cerebrospinal fluid have found immune signatures specific to different stages of multiple sclerosis (MS). These signatures can predict treatment responses and identify MS subtypes [48,49]. Relapsing-remitting MS shows increased activated $CD4^+$ T-cell populations that express gene programs linked to interferon-γ (IFN-γ). In contrast, progressive MS has more cytotoxic $CD8^+$ T cell infiltration and distinct inflammatory microglial activation patterns related to chronic neurodegeneration [50,51]. These single-cell features have clear therapeutic implications. Patients with IL-17-driven gene programs tend to respond better to IL-17-targeting treatments, while those with IFN-γ-dominant immune profiles usually respond more favorably to interferon-based therapies [52]. ScRNA-seq studies of immune cells in autoimmune encephalitis have revealed significant differences among the various illness types. Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is mainly characterized by the infiltration of B cells and plasma cells, with evidence of ongoing oligoclonal antibody production in the central nervous system [53,54]. The differences in response to B-cell-focused versus T-cell-targeted treatments can be explained by other types of autoimmune encephalitis that show T-cell-dominant infiltrates [55]. Overall, our findings highlight how scRNA-seq could help advance autoimmune neurology towards treatments guided by immune phenotypes.

Current limitations and future directions

Despite its revolutionary potential, scRNA-seq has several significant drawbacks. Data can be biased toward cell types that are easier to dissociate or more active in transcription. This happens because capture efficiency is still very low, often sampling only 5 to 10% of cells from complex tissues [56]. Additionally, errors from enzymatic tissue separation can alter transcription patterns significantly, particularly in delicate populations like microglia and tissue-resident macrophages [57]. Moreover, integrating data across studies and ensuring repeatability are still challenged by batch effects caused by differences in sample processing, reagent lots, sequencing depth, and computational methods [58]. Future methodological advances are therefore focused on four major areas.

- Combining transcriptomics with proteomics, epigenomics, and chromatin accessibility through multi-omics approaches.
- Integrating spatial transcriptomic data to maintain anatomical context.
- Creating standardized experimental and analytical pipelines to facilitate extensive meta-analyses across institutions.
- By advancing dissociation chemistry and nucleus-based sequencing techniques, scRNA-seq technologies can be extended to technically difficult tissues such as bone and mineralized cartilage [59,60].

Spatial transcriptomics: Visualizing inflammation in its tissue context

By maintaining two- or three-dimensional tissue architecture while concurrently recording gene expression patterns, spatial transcriptomics overcomes a major constraint of scRNA-seq and permits direct observation of pathogenic immune cells inside their natural milieu [61]. This method tackles a crucial concern in autoimmune neurology: how local tissue environment influences immune-mediated harm, as well as which cells are pathogenic and where they function.

Technical platforms and methodologies

Imaging-based and sequencing-based approaches are the two main types of spatial transcriptomic platforms. Although they have limited transcript coverage (about 500–5,000 genes), imaging-based techniques like seqFISH, MERFISH, and RNAscope reach near-nanoscale precision by using multiplexed fluorescent probes that target specified RNA sequences [62,63]. Genome-wide transcriptome profiling at resolutions of around 50–100 microns is made possible by sequencing-based systems like Visium (10x Genomics) and Stereo-seq, which collect RNA onto spatially barcoded surfaces [64,65]. These alternative approaches allow for customized application based on biological issues by striking a balance between transcriptome breadth and spatial resolution.

Applications in neuroinflammation

Spatial transcriptomics has uncovered a hitherto overlooked spatial arrangement of inflammation in multiple sclerosis that explains disease progression patterns. Meningeal immune complexes in actively demyelinating lesions send gradients of inflammatory signals into nearby cortical gray matter, which triggers complement activation, especially in the terminal complement cascade, and eventual neurodegeneration [66,67]. These results give treatment approaches that target complement components in progressive multiple sclerosis a molecular basis. Emerging spatial transcriptomic investigations are starting to map how antibodies that target neuronal antigens (such as NMDAR, AMPAR, and GABA receptors create localized inflammatory microenvironments that affect neuronal recovery and functioning in autoimmune encephalitis [68]. According to preliminary data, the severity of the disease is correlated with the close geographical proximity of neurons expressing target antigens and antibody-producing B cells, which supports treatment strategies meant to interfere with immune cell trafficking to afflicted areas [69].

CRISPR screens: From observation to causation

Genome-wide association studies do not prove functional

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causation, but they do uncover genetic risk loci for autoimmune neurological disorders. By allowing the systematic disruption of thousands of genes to directly evaluate their functions in immune regulation, pathogenic signaling, and therapeutic response, CRISPR-based screening tools fill this gap [70].

Pooled and focused CRISPR approaches

In autoimmune neurology, CRISPR-based functional genomic screens are becoming essential for moving from genetic connections to identifying gene function. Libraries with about 20,000 to 25,000 single guide RNAs (sgRNAs) target nearly all protein-coding genes and are introduced into large cell populations during pooled CRISPR screenings [71]. Typically, researchers use lentiviral vectors to ensure steady expression. Each cell receives one sgRNA, enabling high-throughput genome-wide loss-of-function testing. After genetic disruption, cells face disease-related selection pressures, including cytokine stimulation (like IL-2 or IL-7 for T-cell activation), antigen exposure, or inflammatory stress. In these situations, cells with sgRNAs targeting genes essential for survival or activation are likely to be eliminated [72,73]. In contrast, cells with sgRNAs that block genes preventing harmful traits survive and become more abundant. By deep sequencing the sgRNA representations before and after selection, researchers can identify genes that control immune activation, differentiation, or tolerance. CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi) technologies allow for controlled upregulation or downregulation of gene expression without creating irreversible DNA breaks, improving on traditional knockout methods [74-76]. These epigenetic disruption techniques are particularly valuable for finding therapeutic targets that require partial modulation instead of total suppression since they enable precise adjustments in gene activity. Recent CRISPRa/i screens have pointed to new molecular pathways that might help restore immune tolerance in autoimmune neurological disorders by uncovering previously unknown regulators of regulatory T-cell (Treg) development, stability, and suppressive activity.

Target discovery in autoimmune neurology

CRISPR screening of disease-related cellular models has given clear molecular insights into autoimmune neurological diseases. Genome-wide CRISPR screens in human induced pluripotent stem cell (iPSC)-derived microglia have revealed that genetic differences in the multiple sclerosis (MS)-related region MS4A4A specifically influence microglial reactions to myelin debris. Reducing MS4A4A through CRISPR lowered pro-inflammatory cytokine production and hindered phagocytosis [78]. This linked GWAS-identified risk areas to microglial dysfunction and ongoing neuroinflammation. These findings indicate that MS4A4A could be a potential target for changing innate immune responses in multiple sclerosis. In B-cell lines from myasthenia gravis (MG) patients, targeted CRISPR screens have found genes who's silencing significantly cuts down the production of anti-acetylcholine receptor (AChR) antibodies. Among these, the CD180 (RP105) gene stood out as a key regulator of B-cell activation in MG. Interestingly, while CRISPR-mediated deletion of CD180 unexpectedly increased antibody production, boosting CD180 signaling through drugs or genetics reduced autoreactive B-cell responses. This surprising result shows how functional genomics can help find therapeutically relevant targets that may not be obvious from genetic association studies alone [79].

Therapeutic Applications: From Discovery to Clinical Translation

Advances in single-cell genomics and CRISPR-based functional screening have spurred a new class of precision immunotherapies targeted at selectively eliminating or reprogramming pathogenic immune populations while preserving protective immunity [80].

CAR-T cell therapy: Resetting autoreactive B cells

Chimeric antigen receptor (CAR) T-cell therapy, which was first developed to treat hematologic malignancies, has emerged as one of the most innovative treatment modalities for severe and refractory autoimmune neurological disorders [81]. CAR-T cells, which are autologous T lymphocytes genetically altered ex vivo to generate synthetic receptors that recognize specific antigens on target cells, enable highly selective immune-mediated cytotoxicity [82].

Mechanism and engineered variants

By identifying surface antigens such as CD19, which is extensively expressed throughout B-cell embryonic stages, and BCMA (B-cell maturation antigen), which is exclusively expressed on long-lived plasma cells, CAR-T methods in autoimmune neurology have mostly targeted pathogenic B-cell populations [83]. After infusion, CAR-T cells identify antigens on target cells and cause apoptosis by releasing cytotoxic effector molecules such as granzyme B and perforin, as well as pro-apoptotic cytokines [84]. Bispecific CAR-T cells with dual antigen targeting capabilities, such as combination CD19 and BCMA recognition, have been developed recently [85]. This method allows for the simultaneous eradication of long-lived antibody-secreting plasma cells ($BCMA^+$) and naïve and memory B cells ($CD19^+$), which is a significant conceptual advancement for autoimmune illnesses. Bispecific CAR-T treatments accomplish a more thorough "immune reset" by eliminating both tissue-resident plasma-cell reservoirs and active antibody production, potentially providing long-lasting, medication-free remission in refractory autoimmune neurological diseases [86].

Clinical efficacy in Myasthenia gravis

The first strong evidence that cellular immunotherapy can lead to significant and lasting remission in antibody-mediated neurological disease came from a key phase 1 clinical trial evaluating anti-BCMA/CD19 bispecific CAR-T cells in patients with hard-to-treat generalized myasthenia gravis (MG) [86]. The study included 18 patients with severe symptoms (MG-ADL ≥6) who had not responded to several prior treatments, including corticosteroids, non-steroidal immunosuppressants, rituximab, plasma exchange, and intravenous immunoglobulin [87]. By day 180 after receiving a single CAR-T cell injection, 82% of patients reached minimum manifestation status, which is a level of illness control rarely seen in refractory MG patients [88]. Remarkably, 100% of patients stopped using non-steroidal immunosuppressive medications, and 88% stopped using all glucocorticoids. This highlights the therapy's potential for reducing steroid use and providing lasting disease modification [89]. Importantly, total seronegative conversion of anti-acetylcholine receptor (AChR) antibodies occurred in 47% of treated patients. This finding is unusual with traditional immunotherapies and indicates that autoreactive B-cell and plasma-cell populations have been largely eliminated [90]. The safety profile was positive. There were no indications of

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immunological effector cell-associated neurotoxicity syndrome (ICANS) or dose-limiting toxicities. About 39% of patients experienced grade 1 cytokine release syndrome (CRS), which was managed with supportive care alone [91]. Overall, these results indicate that a single cell-based treatment can lead to lasting, medication-free remission in patients previously considered treatment-resistant. This represents a significant shift in the approach to treatment.

Multiple sclerosis and NMOSD

CD19-directed CAR-T cell treatments are presently being studied in both relapsing-remitting and progressive multiple sclerosis (MS) (NCT06384976), motivated by success in MG [92]. According to preclinical research, CAR-T cells may successfully eliminate pathogenic B-cell populations in active CNS lesions by passing across the blood–brain barrier [93]. Longer-term follow-up and phase 3 validation are still awaited; early phase 1/2 clinical observations show decreases in MRI lesion load and inflammatory activity [94]. Pathogenic anti-aquaporin-4 (AQP4) antibodies cause complement-mediated astrocyte damage in neuromyelitis optica spectrum disease (NMOSD). The goal of CD19-directed CAR-T cell therapy (NCT05828212) is to eradicate the B-cell populations that produce antibodies over time [95]. Although official efficacy results have not yet been released, preliminary clinical findings indicate biological activity and potential therapeutic benefit [96].

Beyond antibody depletion: Mechanistic insights into FcRn blockade

Despite the fact that FcRn antagonists were once thought of as straightforward IgG-lowering medications, mounting data indicate that their therapeutic effectiveness goes beyond quantitative antibody depletion. In addition to endothelial cells, antigen-presenting cells (APCs) such as dendritic cells, macrophages, and B cells also express FcRn. Blocking FcRn has wider immunomodulatory effects by changing immune complex trafficking, decreasing the effectiveness of antigen presentation, and attenuating downstream T-cell activation [60–62]. Early in the course of myasthenia gravis (MG), quick elimination of pathogenic antibodies may prevent complement activation and neuromuscular junction damage, allowing postsynaptic folds and acetylcholine receptor density to recover structurally. This might account for the long-lasting clinical effect seen even when medication is stopped, and IgG levels return [63,64]. Furthermore, high-affinity, pathogenic IgG subclasses (IgG1 and IgG3), which are primarily in charge of complement-mediated tissue damage, are selectively reduced by FcRn inhibition [65]. Additionally, new research suggests that FcRn antagonists promote a tissue-level mechanism different from systemic immunosuppression by reducing intramuscular immune complex deposition and dampening local inflammatory signals at the neuromuscular junction [66].

FcRn inhibitors across autoimmune neurological diseases

FcRn inhibitors are being tested for a variety of antibody-mediated neurological conditions in addition to MG. Efgartigimod received regulatory clearance in 2024 after showing notable improvements in nerve conduction measures and disability ratings in patients with chronic inflammatory demyelinating polyneuropathy (CIDP) [67]. FcRn blockage has been validated as a platform immunotherapy for autoantibody-driven disorders by similar advantages shown in immunological thrombocytopenia (ITP) [68]. FcRn inhibition is a strong supplement or substitute for complement inhibition in neuromyelitis optica spectrum disease (NMOSD). FcRn antagonists may stop astrocytic damage and disruption of the blood-brain barrier during acute relapses by quickly reducing circulating anti-AQP4 antibodies. This approach is presently being tested in phase 2 studies, and preliminary results point to a decrease in recurrence without a higher risk of infection [69,70]. FcRn inhibitors may be involved in antibody-dominant subtypes of multiple sclerosis (MS), such as pattern II demyelination, where humoral immunity and complement activation are significant, although they are unlikely to replace disease-modifying treatments [71].

Comparative positioning of emerging immunotherapies

A logical framework for therapy selection is required due to the growing therapeutic landscape in autoimmune neurology. The mechanisms, durability, risk profile, and cost of complement inhibitors, FcRn antagonists, bispecific antibodies, and CAR-T treatments are substantially different. Although complement inhibitors offer quick symptom relief, they must be used continuously and do not stop the formation of autoantibodies upstream [72]. FcRn antagonists are appropriate for both early illness intervention and chronic maintenance because they provide selective, reversible immunomodulation with exceptional safety [73]. In the center, bispecific antibodies allow for scalable production and focused immune cell activation, but they also need close safety monitoring [74]. By eliminating autoreactive B-cell clones, CAR-T cell treatment is a potentially therapeutic approach that can induce long-term, drug-free remission. However, because of its expense, complexity, and uncertain long-term dangers, its usage is now restricted to severe, refractory diseases [75–77].

Future directions and translational challenges

Precision immunotherapy based on immunophenotypic, genetic, and serological biomarkers is the way of the future for autoimmune neurology. For the best treatment matching, stratification according to antibody subtype, complement activation state, and B-cell lineage participation will be crucial [78]. A potential area is combination treatments, such as CAR-T therapy for long-lasting immune reprogramming after FcRn antagonists for quick antibody removal. Safety and accessibility may be significantly increased by developments in controlled CAR constructions with suicide switches and allogeneic "off-the-shelf" CAR-T platforms [79–81]. There are still a lot of regulatory obstacles. Adaptive clinical trial designs and prolonged post-marketing surveillance are necessary due to novel mechanisms, customized manufacturing, and long-term immunological effects. However, the effectiveness of these treatments represents a clear transition from nonspecific immunosuppression to mechanism-driven immune reset [82–86].

Emerging Biotechnologies and Future Directions

Recent developments in biotechnology are changing how autoimmune neurological disorders are understood and treated. Important developments include tissue-engineered organoid models, engineered regulatory T cells, nanoparticle-based CNS delivery, and artificial intelligence.

Artificial intelligence and machine learning for disease prediction and biomarker discovery

In autoimmune neurology, the use of artificial intelligence (AI)

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and machine learning (ML) for early diagnosis, patient classification, and treatment prediction is growing [97].

MRI-based diagnosis

AI algorithms have been created to examine hippocampus anatomical patterns in MRI images of individuals suspected of having autoimmune encephalitis [98-100]. AI models predicted anti-NMDAR antibody presence with 89% accuracy in a multicenter trial from Germany and Switzerland, potentially enabling therapy commencement weeks prior to test confirmation [101]. This illustrates how AI can extract minute imaging details that are not apparent to human observers and convert them into data that can be used for therapeutic purposes.

Immunological phenotyping and treatment prediction

In order to predict treatment response, machine learning algorithms that integrate scRNA-seq, proteomics, and genomic data can uncover immune signals unique to each patient. For example, patients with IL-17-dominant profiles benefit better from IL-17-targeted therapy, whereas those with IFN-γ-dominant profiles respond preferentially to interferon-β. When prospectively confirmed, these prediction models might facilitate precision medicine, maximize treatment, and reduce needless immunosuppression [102].

Drug repositioning and target discovery

AI-driven medication repositioning finds potential treatments by utilizing gene-expression datasets, chemical structures, and literature. AI can speed up target identification and repurpose existing medications, as evidenced by recent analysis that identified CRBN-targeting molecules as possible autoimmune encephalitis therapies [103].

Nanoparticle delivery systems for CNS targeting

For CNS-targeted therapies, the blood-brain barrier (BBB) is still a significant obstacle. Nanoparticle-based methods enable the selective delivery of nucleic acids, biologics, and small compounds.

Liposomal and polymeric nanoparticles

To improve BBB penetration, lipid nanoparticles (LNPs) containing siRNA against pro-inflammatory cytokines (IL-17, TNF-α, and IL-6) have been functionalized with transferrin or RGD peptides. LNP-siRNA was more successful than systemic treatment in reducing CNS inflammation and demyelination in EAE animals [104].

Using a technique known as therapeutic tolerance, polymeric nanoparticles containing myelin peptides target lymph nodes to increase regulatory T cells (Tregs). Proof-of-concept for immune regulation was demonstrated by liposomal formulations that delivered myelin antigens with IL-10 and TGF-β, preventing the development of EAE [105].

Biohybrid approaches

Red blood cell membrane-coated particles coupled with immunosuppressive compounds are examples of biohybrid nanoparticles that increase CNS transport and avoid immune detection, providing a flexible tool for targeted treatment [106].

Engineered regulatory T cells and CAR-Treg therapy

Instead of eradicating harmful cells, CAR-Treg treatment uses modified Tregs to restore immunological tolerance.

Concept and rationale

Autologous Tregs that express chimeric antigen receptors (CARs) specific to antigens linked to illness are known as CAR-Tregs. Unlike traditional CAR-T cells that destroy target cells, they multiply and produce TGF-β and IL-10 upon antigen interaction, causing tissue healing and localized immunosuppression [107].

Neurological applications

CAR-Tregs that target AChR epitopes stopped the development of EAE and reversed active illness in preclinical models of myasthenia gravis, indicating the possibility of long-lasting tolerance [102]. Low circulating Treg frequencies (~5% of CD4+ T cells), preserving Treg phenotype ex vivo, and guaranteeing antigen specificity to prevent unwanted immunosuppression are challenges.

Tissue engineering and organoid models

Compared to 2D cultures, three-dimensional organoid models more closely mimic real neuroinflammation, enabling mechanistic research and therapeutic testing.

Neuroimmune organoids

To observe T- and B-cell invasion, microglial activation, and neuronal dysfunction, patient-derived immune cells can be co-cultured with iPSC-derived organoids that include neurons, glia, microglia, and endothelium. The platform's usefulness for tailored therapeutic screening was demonstrated by the dose-dependent neuronal damage caused by anti-NMDAR antibodies, which was reversible by B-cell depletion [108].

Gut-on-a-chip models

The gut-brain axis may be studied using gut-on-a-chip technologies, which show how intestinal barrier disruption or microbial dysbiosis drives systemic immune responses. Interventions that prevent downstream CNS autoimmunity and restore barrier function are being screened using these models [109].

Current Challenges and Limitations

Translating discovery to clinical practice

The "valley of death" between preclinical discovery and clinical implementation is a major obstacle in autoimmune neurology. Although rare pathogenic cell subsets can be found using technologies like single-cell RNA sequencing (scRNA-seq), turning these discoveries into therapeutic targets necessitates a number of validation steps, such as CRISPR-based functional assays, animal model testing, and phased human trials, which frequently take five to ten years [110]. Furthermore, there is significant variation in autoimmune neurological disorders; for example, a pathogenic B-cell subset observed in 50% of individuals with myasthenia gravis (MG) may not be significant in the remaining patients, leading to inconsistent treatment results. Therefore, although these methods are still in their infancy, biomarker-guided patient classification is crucial for precision medicine [111].

Safety of novel cellular therapies

Even while CAR-T treatments are incredibly effective, long-term safety profiles are still unclear. There are worries regarding late-onset toxicities since CAR-T cells can last longer than five years. Immunodeficiency can result from on-target, off-tissue consequences like normal B cell loss. Large-scale prospective validation is required to verify the reliability of safety switches, such as inducible caspase-9 systems that enable quick CAR-T ablation following unfavorable occurrences [112,113].

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Cost and access

With expenses per patient ranging from $300,000 to $500,000 and production times of 4–8 weeks, CAR-T treatment is still unaffordable. These considerations restrict access, especially in places with limited resources, underscoring the need for universal allogeneic CAR-T methods or other, less expensive treatments. Although they are more widely available, bispecific antibodies, complement inhibitors, and FcRn antagonists might not be as effective as CAR-T treatment [114,115].

Limitations of animal models

Experimental autoimmune encephalomyelitis (EAE), a rodent model of multiple sclerosis (MS), is crucial to preclinical research. But although human MS is multiphasic and increasingly understood to be B-cell mediated, EAE is usually monophasic and T-cell driven. Clinical studies have frequently failed to translate EAE results (e.g., anti-LINGO-1 treatment, anti-VLA-4 beyond natalizumab). Therefore, increasing the prediction accuracy of preclinical research requires more physiologically appropriate models, such as organoids, humanized mice, and organ-on-a-chip systems [116,117].

Clinical Trials and Regulatory Landscape

Current trial landscape

By 2025, more than 70 clinical studies will be examining CAR-T cell treatments for autoimmune illnesses, mostly focusing on multiple sclerosis (MS), myasthenia gravis (MG), neuromyelitis optica spectrum disorder (NMOSD), and systemic lupus erythematosus (SLE) [118]. Among the notable ongoing studies are:

- NCT06384976 (Kymriah): CD19-directed CAR-T in progressive MS (Phase 2, active).
- NCT05828225 (Kymriah): Phase 1, active CD19-directed CAR-T in generalized MG.
- NCT06799247 (Descartes-08): CAR-T guided by BCMA in generalized MG (Phase 3, recruiting).
- NCT05828212: Phase 1 recruitment of CD19-directed CAR-T in NMOSD.

Simultaneously, initiatives for bispecific antibodies are growing; currently, over 15 candidates are being developed to target autoimmune neurological diseases. Together, these studies demonstrate how quickly cellular and antibody-based immunotherapies are being tested in clinical settings [119].

Regulatory considerations

Although the FDA has set CAR-T standards for cancer, it is difficult to apply them to autoimmune diseases. Patients frequently have comorbidities and pre-existing immunosuppression, which calls for rigorous long-term toxicity monitoring [120]. Additionally, endpoint selection, cross-trial comparisons, and meta-analyses are made more difficult by various definitions of clinical response among disorders (e.g., MG, MS, NMOSD). Extended follow-up periods are necessary to determine response durability and optimum outcome metrics (e.g., minimum manifestation status vs. full remission) [121,122].

Future Perspectives and Roadmap

Precision medicine integration

Precision medicine through the integration of multi-omics data with clinical phenotyping is the next frontier in autoimmune neurology. Patients can be categorized into categories most likely to react to certain treatments using prospective biomarker studies, such as scRNA-seq, TCR sequencing, and proteome profiling. Accurate treatment success prediction and customized therapy selection may be made possible by machine learning algorithms that include these biomarkers with neuroimaging and clinical data [123-126].

Combination therapies

Despite the transformational potential of monotherapies, sensible combination tactics may be necessary to achieve full remission. For example, combining tailored Treg treatment (which restores immunological tolerance), CAR-T cells (which target pathogenic B cells), and tolerogenic signals given by nanoparticles may overcome resistance mechanisms and improve results [127-130].

Moving beyond Off-label use toward biomarker-driven development

Endotype-specific therapies should replace general illness classifications in therapeutic approaches. For instance, anti-MuSK+ MG is mostly T-cell mediated, while anti-AChR+ MG is characterized by complement-mediated destruction. Complement inhibitors for anti-AChR+ patients and T-cell depletion for anti-MuSK+ patients are examples of tailored treatment that can improve effectiveness and reduce side effects [130-132].

Long-term follow-up and real-world evidence

Small sample sizes (10–50 patients) and brief follow-up (6–12 months) are the main limitations of current clinical trials. Long-term registry studies that monitor CAR-T and other cutting-edge treatments for five to ten years are essential for assessing late-onset toxicity, safety, and remission duration. Simultaneously, the discovery of predictive biomarkers and patient characteristics that guide therapy will be made easier by real-world evidence (RWE) projects that gather standardized prospective data across various patient group [133,134].

Discussion

Among the most complicated and crippling conditions affecting the nervous system are autoimmune neurological illnesses, which include multiple sclerosis (MS), myasthenia gravis (MG), neuromyelitis optica spectrum disorder (NMOSD), and autoimmune encephalitis (AIE). In the past, broad-spectrum immunosuppression was a major component of treatment plans, which frequently led to long-term morbidity, side effects, and insufficient disease management. This paradigm has been drastically altered by recent biotechnological developments, which make it possible to precisely examine the cellular and molecular mechanisms underlying autoimmunity and provide guidance for the creation of tailored treatments. Unprecedented understanding of immunological heterogeneity, tissue

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localization, and dynamic pathogenic states has been made possible by single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics. These technologies have uncovered transcriptional processes linked to disease activity and treatment response, explained mechanisms of compartmentalized CNS immunity, and found uncommon but clinically significant immune subsets.In addition to these methods, CRISPR-based functional genomics allows for the causal examination of genes linked to disease, revealing useful targets like MS4A4A in MS and CD180 in MG. The development of precision immunotherapies as a therapeutic application represents a revolutionary turning point. In refractory MG, CAR-T cell therapy—especially bispecific anti-CD19/BCMA constructs—has shown long-lasting, drug-free remission, and preliminary clinical data points to its effectiveness in MS and NMOSD. While engineered CAR-Tregs, nanoparticle-based CNS delivery systems, and organoid models increase the potential for restoring immune tolerance and enabling customized therapeutic screening, FcRn inhibitors and complement-targeting agents offer additional tools for quick modulation of pathogenic antibodies. Despite these developments, there are still many obstacles to overcome. Long-term safety monitoring, biomarker-guided patient classification, and strong preclinical validation are necessary for converting mechanistic discoveries into clinically successful therapies. Cellular treatments are not widely available due to their high cost and complicated manufacture, and traditional animal models frequently do not accurately reflect the diversity of human diseases. To optimize efficacy, reduce off-target effects, and improve patient-specific results, future techniques must use multi-omics profiling, AI-driven predictive modeling, and logical combination therapy.

Conclusions

In conclusion, autoimmune neurology is entering a new age due to the combination of biotechnology and precision medicine. In patients who were previously thought to be resistant, mechanism-driven therapy now has the ability to not only manage illness but also cause long-lasting remission and immunological reset. The management of autoimmune neurological diseases is expected to change over the next ten years due to developments in biomarker-guided stratification, combination immunotherapies, and scalable delivery platforms. This will bring us closer to a time when these conditions are manageable, preventable, and possibly curable. The field of autoimmune neurological disorders has reached a period of unparalleled scientific comprehension and innovative treatment. The cellular and molecular mechanisms behind these circumstances have been deciphered via biotechnological platforms such as scRNA-seq, CRISPR screens, spatial transcriptomics, and phage display. Complement inhibitors, FcRn antagonists, bispecific antibodies, and CAR-T cell therapy are now mechanistically focused treatments that let patients who were thought to be incurable achieve drug-free remission. However, there are still obstacles to overcome, such as the delayed and costly conversion of discoveries into treatments, the need for long-term safety monitoring, restricted access, and the need for biomarker-driven categorization due to disease category heterogeneity. The following areas should be prioritized over the next ten years: (1) prospective biomarker studies that identify immune signatures that predict treatment response; (2) the creation of more representative human disease models in place of animal models; (3) logical combination therapies that incorporate complementary mechanisms; (4) long-term safety and efficacy studies; and (5) access expansion through less expensive alternatives and resource-appropriate strategies. There is a reasonable chance that autoimmune neurological illnesses, which were formerly catastrophic and always progressing, may be avoidable and treatable due to the confluence of biotechnology and precision medicine.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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