ImmunoHorizons

RESEARCH ARTICLE

Adaptive Immunity

Comparison of B Cell Variable Region Gene Segment Characteristics in Neuro-autoantibodies

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ABSTRACT

Autoimmune pediatric neurologic diseases have variable phenotypes and presentations, making diagnosis challenging. The pathologic mechanisms are also distinct, including cell-mediated and Ab-mediated autoimmunity, paraneoplastic syndromes, and postinfectious processes. In recent years a number of studies have described the characteristics of the autoantibodies involved in a number of these diseases. Some of the described Abs use a restricted set of variable gene segments. We sought to compare the Ab characteristics of autoantibodies related to some of the more common disorders to discover whether specific Ab signatures are universally associated with neuroautoimmune diseases. We initially performed a literature review to summarize the Ab characteristics of autoantibodies related to some of the more common disorders, including *N*-methyl-D-aspartate receptor (NMDAR) and leucine-rich, glioma-inactivated 1 (LGI-1). Next, we performed data analysis from selected studies that sequenced Ig genes to further characterize NMDAR and LGI-1 autoantibodies including CDR3 length distribution, variable gene sequence usage, and isotype use. We found that CDR3 length of NMDAR autoantibodies was normally distributed whereas the CDR3 length distribution of LGI-1 autoantibodies was skewed, suggesting that there is no global structural restriction on types of autoantibodies that can cause encephalitis. We also found that IgG1–IgG3 were the main NMDAR autoantibody isotypes detected, while IgG4 was the major isotype used in autoantibodies from LGI-1 encephalitis. These findings are useful for our understanding of autoimmune encephalitis and will help facilitate better diagnosis and treatment of these conditions in the future. *ImmunoHorizons*, 2024, 8: 740–748.

INTRODUCTION

Encephalitis refers to a severe inflammatory disorder primarily affecting the gray matter of the brain (1). Clinical features include altered mental status, memory loss, psychosis, seizures, and others, often developing over the span of weeks to months (2). The encephalitides are a category of neuroinflammatory diseases with diverse etiologies including infectious and noninfectious processes. The incidence of encephalitis is 5–10 in 100,000 and has a profound global health burden (3). In the United States, there were 7.3 cases per 100,000 persons during 2000–2010, with the highest incidence in infants <1 y of age (13.5 in 100,000) and the lowest incidence in children between 10 and 14 y of age (4.1 in 100,000) (4). In a large surveillance study (the Encephalitis Project of the Emerging Infections Program and the Centers for Disease Control and Prevention), infectious etiologies were only identifiable in approximately one third of cases studied, suggesting that alternate mechanisms include autoimmunity (5, 6).

Autoantibody associations have long been helpful in diagnosing and classifying forms of autoimmune encephalitis and

Received for publication April 17, 2024. Accepted for publication September 20, 2024.

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This work was supported by a grant from the Wildermuth Memorial Foundation through Variety, the Children's Charity of Buffalo & Western New York, and an Infectious Disease Society of America Microbial Pathogenesis in Alzheimer's Disease Grant.

Abbreviations used in this article: AQP, aquaporin; DM, diabetes mellitus; D1R, dopamine 1 receptor; GABA, γ-aminobutyric acid; GAD65, glutamic acid decarboxylase 65; Glun1, glutamate ionotropic subunit 1; hMOG, human MOG; LGI-1, leucine-rich, glioma-inactivated 1; MOG, myelin oligodendrocyte glycoprotein; NMDA, *N*-methyl-d-aspartate; NMDAR, NMDA receptor; NR-1, NMDAR subunit 1; VGCC, voltage-gated calcium channel; VGKC, voltage-gated potassium channel.

https://doi.org/10.4049/immunohorizons.2400037

ImmunoHorizons is published by The American Association of Immunologists, Inc.

related disorders (1). Cloning and sequence analysis of Abs have been a burgeoning method applied across many infectious and autoimmune phenomena. mAbs can further be developed into therapeutics and be used to identify novel vaccine targets. In the past decade, a number of studies providing fine sequence detail have been pursued in these disorders.

Although targeting of autoantibodies to certain Ags is seen in a number of conditions, most described neuro-autoantibodies are specific to clinical presentation (7–10). Ag targets in limbic encephalitis include γ-aminobutyric acid $(GABA)_B$ and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (11, 12), glutamic acid decarboxylase 65 (GAD65) (13–15), $GABA_A$(16), dipeptidyl-peptidase–like protein (DPPX), glycine α_1 receptor ($Gly\alpha_1R$), metabotropic glutamate receptor subtype 5 (mGluR5), neurexin 3a, antiglial nuclear Ab/SOX1, Hu (ANNA1) (17), CV2/collapsing response mediator protein 5 (CRMP5), and Ma1/Ma2. (18). IgG anti-ganglioside q1b (GQ1b) autoantibodies are highly specific to brainstem encephalitis (19, 20). Other conditions, such as Rasmussen encephalitis, are associated with targeting metabotropic glutamate receptor subtype 3 (mGluR3) and anti-Ri (ANNA2) (21, 22). Several autoantibody targets have been shown in Sydenham's chorea, including anti–dopamine 1 receptor (D1R) and dopamine 2 receptor (D2R), anti-tubulin, anti–ganglioside-monosialic acid 1 (GM1), and calcium calmodulin-dependent protein kinase II (CaMKII) (23). Pediatric acute neuropsychiatric syndrome (also known as pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections) has been associated with autoantibodies to D1R and dopamine receptor 2L (D2L), GM1, calcium calmodulin-dependent protein kinase II (CaMKII) and tubulin (24), although the clinical utility of these remains controversial (25). Even autism spectrum disorder has been associated with autoantibodies to myelin proteins, serotonin receptors, brain endothelium, cerebellar tissue, and glutamic acid decarboxylase as well as to as to more ubiquitous proteins such as folate receptor α and mitochondria (26).

Of the Ags targeted in multiple conditions, aquaporin (AQP), myelin oligodendrocyte glycoprotein (MOG), and voltage-gated calcium channels (VGCCs) are the best studied. AQP4 is targeted in diencephalic, brainstem, and encephalomyelitis. MOG is targeted in cortical, brainstem, and encephalomyelitis and can be seen in monophasic demyelinating events such as acute disseminated encephalomyelitis (27, 28). Global targeting of VGCCs, also found in autism spectrum disorder (29), may be a nonspecific marker of brain inflammation as they are seen in a broad range of conditions (30). Autoantibodies against the voltage-gated potassium channels (VGKCs) have been reported in a number of neurologic conditions affecting both children and adults, including limbic encephalitis, seizure disorders, acute disseminated encephalomyelitis, and Morvan's syndrome (31, 32). Interestingly, specific components of the VGKC complex, such as leucine-rich, glioma-inactivated 1 (LGI-1) and contactin-associated protein like 2, are major targets of VGKC complex autoantibodies in adults with LGI-1 having specific association with limbic and striatal encephalitis (33–35). Recent studies have characterized a number of V region sequences from LGI-1 autoantibodies (36, 37).

In 2007, a sentinel study was published documenting a novel form of autoimmune encephalitis, anti–*N*-methyl-D-aspartate receptor (NMDAR) encephalitis (38), originally described as a paraneoplastic syndrome associated in women with ovarian cancer. Shortly afterward, anti-NMDAR encephalitis was also identified in a relatively large number of previously unexplained cases in the California Encephalitis Project (39, 40). Today, it is known to be the most common cause of autoimmune encephalitis in children. Anti-NMDAR encephalitis presents with a wide variety of neurologic deficiencies, including abnormal behavior, memory deficits, seizures, and abnormal movements (41). Children seem to develop seizures more frequently, whereas adults develop psychiatric abnormalities. CSF IgG autoantibodies targeting the glutamate ionotropic subunit 1 (GluN1) subunit of the NMDAR play a major role in the pathogenesis (42).

The binding area of an Ab to its epitope is in the V region which, for the Ab H chain, is derived from transcripts from the V, D, and J genes. During B cell development, generally a single representative of the roughly 65 V genes, 27 D genes, and 6 J genes is genetically recombined to provide subsequent unique Ab transcripts to each B cell. In recent years, a number of studies have described the characteristics of the autoantibodies involved in a number of these diseases. Some of these were targeted by Abs using a very restricted set of V genes. Nonetheless, our global understanding of autoimmune encephalitis and the rules that govern neuro-autoantibody pathogenesis remain poorly understood. We sought to compare the Ab characteristics that target autoimmune Ags in the CSF to discover global shared or differentiating characteristics that can be exploited for improved diagnostic and therapeutic targets. As some of the more extensive descriptions of the V region characteristics of neuro-autoantibodies have been against the LGI-1 and NMDA Ags, we initially focused on comparing and contrasting the targeting of these two Ags.

MATERIALS AND METHODS

This study sought to review the literature to summarize the Ab characteristics of autoantibodies related to autoimmune neurologic disease. A PubMed search was performed to identify the studies that isolated mAbs associated with neurologic autoimmune diseases utilizing the following keywords: B cell repertoire, autoimmune neurologic diseases, neuroimmune Abs, anti-NMDA, anti-AQP4, anti-MOG, anti–LGI-1, anti-GAD65, CaMKII, VGCC, anti-GQ1b, GABA, AMPA, ANNA1, CRMP5, D1R, and D2R. We further compared NMDAR and LGI-1 autoantibodies, as anti-NMDAR and limbic encephalitis are the two most common causes for Ab-mediated encephalitis and there were robust datasets from recent human studies available. We specifically collected data on isotype usage, V usage, and V region CDR3 amino acid length. To avoid overrepresentation bias that can occur when reviewing individual sequences, we based our

https://doi.org/10.4049/immunohorizons.2400037

analysis on clonal groups unless specifically noted. Data from these studies used international ImMunoGeneTics information system (IMGT) nomenclature. GraphPad prism 9 was used to create graphs and to analyze normal distributions of the CDR3 lengths using the Anderson–Darling test, D'Agostino–Pearson test, and Shapiro–Wilk test.

RESULTS

A number of recent studies have described the Ig gene characteristics (V usage, D segment usage, J chain usage, mutation rates, CDR3 sequences) for autoimmune encephalitides. To delineate characteristics that can be exploited for improved diagnostic and therapeutic targets, we sought to compare Ab characteristics among the most common classes of autoimmune encephalitis. In performing our literature search, 60 studies were reviewed and 10 studies published the V region sequence data and are summarized in Table I.

MOG and GAD65 autoantibodies

A number of animal model studies have explored repertoire usage after vaccination (43). After mice were immunized by recombinant human MOG (hMOG), 15 hybridomas were identified that secreted mAbs with specific recognition of recombinant hMOG. None of the mAbs shared both V_H and V_L domain sequences. Four of the Abs were IgG2b isotypes, and the remaining were IgG1. The study demonstrated that

TABLE I. Summary of studies that isolated Ab sequences associated with neurologic autoimmune diseases

Study	Focus	Ag Target	Method	Source (human; *nonhuman*)	Ab Sequences Published	Abs Binding Ag Target	Published HCDR	Characterization
Sharma et al. (55)	NMDA receptor	GluN1 (Nr-1) subunit	Hybridoma	PBMCs	Yes	2 (5F5 and 2G6)	n/a	Heavily mutated, long CDR3s
Kreye et al. (53)	NMDA receptor	GluN1 (NR-1) subunit	Single cell cloning	CSF	141 clones (170 total)	6 clones (9 total Abs)	Yes	No variable chain bias noted; low number of somatic hypermutation
Feng et al. (54)	NMDA receptor	GluN1 (NR-1) subunit	Single cell isolation: flow cytometry	CSF	44 clones	42 clones	Yes	Low mutation rate; HC bias to use V1, D1, and J3 and avoid V6 and J1; four clones were shared, with ARVGSKYGFETFDI found in 11 of 12 individuals
Bennett et al. (52)	AQP4	AQP4	Single cell isolation: flow cytometry	CSF	93 clones	6 (11 total Abs)	Yes	93 clones (11 recombinant Abs expressed; 6 AQP4 specific); V_H2 and V_H4 bias; high intraclonal diversity
Bansal et al. (43)	MOG	MOG	Hybridoma	*Murine: spleens, LNs, and bone marrow*	Yes	15 Abs	Yes	Multiple epitopes targeted
von Budingen et al. (44)	MOG	MOG	Combinatorial IgG: Fab library	*Marmoset: bone marrow and spleen*	n/a (GenBank)	60 clones	Yes	V_H1, V_H3, KV1, and KV3 skewing
Raju et al. (51)	GAD65	N-GAD65	Humanized Fab	*Humans and mice: serum and CSF*	Yes (total not available)	1 Ab	Yes	14 monoclonal and polyclonal Abs (1 humanized N-GAD65 Ab)
Hampe et al. (50)	GAD65	N-GAD65	Hybridoma	*Mice*	Yes	1 Ab	Yes	Target 4–22 N-terminal of GAD65
Kornau, et al. (36)	LGI-1	LGI-1	Single Cell isolation: flow cytometry	CSF	85 Abs	26 Abs	Yes	85 Abs (from 82 clones) were screened: highly mutated
Ramberger et al. (37)	LGI-1	LGI-1	Single cell isolation: fluorescent foci method	PBMCs	14 clones	14 Abs	Yes	9 bound to LRR and 5 to EPTP domain

Italicized text marks *nonhuman* studies. EPTP, epitempin-repeat; LN, lymph node; LRR, leucine-rich repeat.

hMOG-specific mAbs targeted multiple epitopes instead of one specific region of MOG (43). Another study used a combinatorial IgG-Fab library derived from a marmoset vaccinated with MOG to characterize anti-MOG Ab responses. The study observed use of a limited repertoire of H chain (IGHV1/IGHV3) and L chain genes (IGKV1/IGKV3) in the marmoset anti-MOG Ab repertoire (44).

Autoantibodies to GAD65 have been found in several autoimmune diseases such as stiff person syndrome (45), autoimmune polyendocrine syndrome type 1 (45), Grave's disease (46), and type 1 diabetes mellitus (DM) (47). However, type 1 DM shows the strongest association with GAD65 Abs, and anti-GAD65 Abs can be detected in 70–80% of newly diagnosed children (47). GAD65 Abs in type 1 DM are predominantly directed to conformational epitopes located at the middle and C-terminal part of GAD65 (48). The N-terminal end of the protein does not carry epitopes recognized by sera from type 1 diabetes patients (49). Two mAbs generated by hybridoma (50) and a constructed humanized N-GAD65 mAb (51) recognize a linear epitope located at aa 4–22 of GAD65. This linear epitope was targeted, as it was not a GAD65 epitope associated with type I DM. For AQP4, only one study was available, which showed a remarkable degree of intraclonal diversity among the V_H repertoire and CDR3 length. There was preferential use of V_H2 family germline sequences followed by V_H4 (52). Overall, with both linear and structural epitopes targeted and general diversity in V region structure, there was not a specific autoantibody signature noted.

Comparing NMDA autoantibodies to LGI-1 autoantibodies

A number of studies published full sequence data from human samples related to anti-NMDAR and limbic encephalitides, two of the most common causes for Ab-mediated encephalitis. Due to these robust datasets, we further analyzed these NMDA and LGI-1 autoantibodies to explore whether these encephalitides shared V region repertoire characteristics. In response to Ags, stimulated B cells undergo somatic hypermutation as they expand and beget progeny. Resulting progeny will contain highly similar Ab sequences, and these together are referred to as clones. Clonal cells are noted by the same predicted V usage, same length, and similar sequence of CDRs 1, 2, and 3. To avoid overrepresentation bias that can occur when reviewing individual sequences, we based our analysis on clonal groups unless specifically noted.

NMDA autoantibodies

Three main studies were found that described V region sequence data in detail for NMDA autoantibodies (Table I). NMDAR subunit 1 (NR-1) is the main binding site of NMDA autoantibodies on the NMDA receptor. The first study generated 170 mAbs representing 141 clonal groups identified by a single-cell cloning method from CSF memory B cells and Ab-secreting cells obtained from eight patients diagnosed with anti-NMDAR encephalitis (53). From this, nine Abs representing six clonal groups were NR-1 reactive. The second study used flow cytometric single-cell isolation from the CSF of 12 Chinese patients with anti-NMDAR encephalitis and characterized 92 cells representing 44 clonal groups. Forty-two of these clonal groups were specific in those with NR-1 activity (54). Notably, a common clone with CDR3 ARVGSKYGFETFDI using V segment 1–18 and J segment 3 was seen in 11 of the 12 patients (representing 38% of total Abs). This H chain common clone was not found in datasets of healthy people or patients with anti–LGI-1 encephalitis, multiple sclerosis, or neuromyelitis optica spectrum disorder. The third study used a hybridoma method to produce two unique mAbs from PBMCs of a female patient with anti-NMDAR encephalitis (55). We analyzed the distribution of Ab attributes by clonal group, as analysis by all sequences would show a bias toward commonly used clones.

CDR3 length. CDR3 is the most V region of the Ig and it contributes significantly to the diversity of Ag specificities. Both combinatorial diversity and somatic hypermutation contribute to the high level of variability in this region. The NMDA autoantibodies mean CDR3 length was 15.17 (SD 3.7) (Fig. 1). For those Abs targeting NR-1, the mean length of 14.74 (SD 3.43) was similar to the non–NR-1 targeting Abs of 15.3 (SD 3.82). In random peripheral B cells, CDR3 usage usually follows a normal distribution around the mean. The NR-1–reactive clones showed more variability in CDR3 length usage, failing all tests of normality with particularly lower than expected number of clones using CDR3 length of 14 aa and the distribution. Overall, the total NMDA distribution was "normal" per D'Agostino–Pearson testing ($p = 0.1677$, $\alpha = 0.05$), but failed the

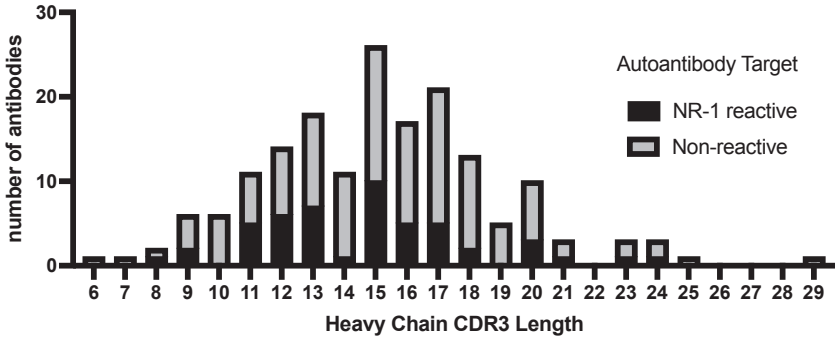

FIGURE 1. NMDA autoantibodies H chain CDR3 length distribution.

NR-1–reactive clones (black) and nonreactive clones (gray) are presented as a portion of the total clones identified.

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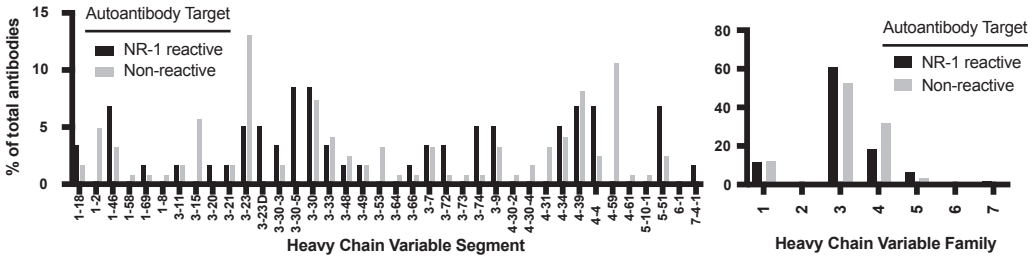

FIGURE 2. NMDA autoantibodies IGHV clonal gene family use.
V gene sequence of clones was performed and sequences counts were graphed by predicted V usage as percentile of total sequences within each subgroup, nonreactive (gray) and NR-1 reactive (black).

Anderson–Darling test ($p = 0.0032$, $\alpha = 0.05$) and Shapiro–Wilk test ($p = 0.0041$, $W = 0.8644$).

Ab gene family usage. The roughly 65 functional H chain Vs can be grouped by similar sequence signatures into seven families (IGHV1–7). For these NMDA-related Abs, the H chain gene usage IGHV3, IGHJ4, and IGHD3 were the most frequently detected. Ab gene families IGHV2 and IGHV6 were not detected. When comparing both groups, Ab gene family IGHV7 and IGHD7 did not appear in the nonreactive group. Fig. 2 shows the IGHV family genes observed.

Isotype use. From the total Abs described, IgG represented 70.13% of the generated Abs followed by IgA (17.36%) and IgM (12.5%). Among the IgG group, IgG1 was the most abundant, followed by IgG2 and IgG3 (Fig. 3). Isotypes were not reported on all sequences. Of the nine Abs that were NR-1 reactive with isotype reporting, all were IgG (66.66% were IgGI). IgG Abs were the most abundant in the nonreactive group (68.29%), followed by IgA (19.51%) and then IgM (12.19%). Of the nonreactive clones, the distribution was comparable to other studies of isotype usage in random Abs.

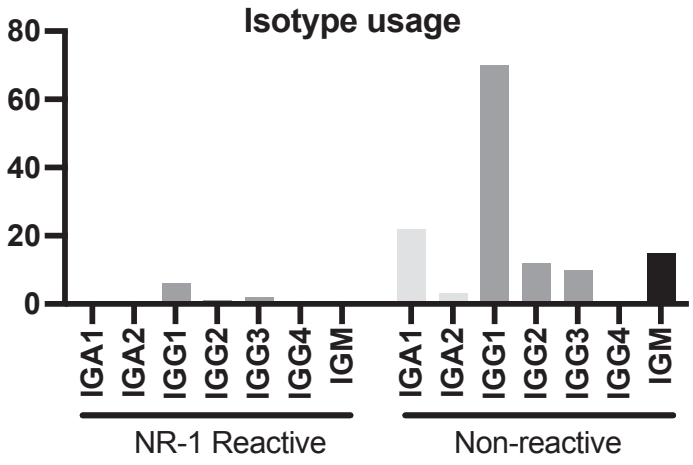

FIGURE 3. NMDA autoantibody Ig isotypes delineated by NR-1 reactivity.
NR-1–reactive Abs (left side) and NR-1 nonreactive Abs (right side) are shown. Few of the described NR-1–reactive clones had specified isotypes identified. Of those identified, all were IgG (dark gray), with 67% being IgG1. IgG Abs were the most abundant in the NR-1 nonreactive group (68%), with most of these being IgG1, followed by IgA (20%; light gray) and IgM (12%; black).

LGI-1 autoantibodies

LGI-1 autoantibodies were cloned and reported by two studies. Kornau et al. (36) isolated 85 different LGI-1 autoantibodies (from 82 clones) from CSF of three patients with LGI-1 encephalitis using single-cell isolation and flow cytometry. These Abs were tested for their reactivity to LGI-1, and 26 were reactive. Ramberger et al. (37) generated 14 different LGI-1–reactive mAbs from peripheral B cells of two patients with limbic encephalitis using single-cell isolation and the fluorescent foci method. Abs generated from the two studies were highly diverse and had no shared Ab sequences between them. Data from the two studies were analyzed in terms of CDR3 length, Ab H chain gene family use, isotype use, and clonality and then further classified and described based on their reactivity to LGI-1.

CDR3 length. The mean CDR3 length was 16.22 aa (SD 3.76). LGI-1 autoantibodies with a CDR3 length of 13 aa accounted for the largest proportion (Fig. 4). Reactive clones had a mean length of 17.17 aa (SD 3.95) compared with 15.57 aa (SD 3.52) in the nonreactive ones. We evaluated normality of CDR3 lengths using the Anderson–Darling test ($p = 0.0013$, $\alpha = 0.05$), D'Agostino–Pearson test ($p = 0.0168$, $\alpha = 0.05$), and Shapiro–Wilk test ($p = 0.0010$, $W = 0.8315$) and none showed normal distribution. The nonnormative distributions and CDR3 length were markedly contrasted to NMDA autoantibodies and NR-1 reactive specifically (Fig. 1).

Ab gene family usage. The H chain gene usages most frequently detected were IGHV3, IGHJ4, and IGHD3 (H chain V usage shown in Fig. 5). Both groups lacked Abs that used members of V family 6 and 7, and nonreactive clones also lacked any described clones utilizing V family 2.

Isotype use. IgG represented 57% of the generated Abs followed by IgM (33.33%) and then IgA (9.37%). Among the IgG group, IgG1 comprised 21.8% of the total IgG generated Abs, IgG2 25.45%, IgG3 1.81%, and IgG4 50.9%. The reactive clones were IgG only (IgG1 15.38%, IgG2 23.07%, and IgG4 61.53%). IgM represented 56.14% of the nonreactive clones followed by IgG (28.07%) and then IgA (15.78%) (Fig. 6).

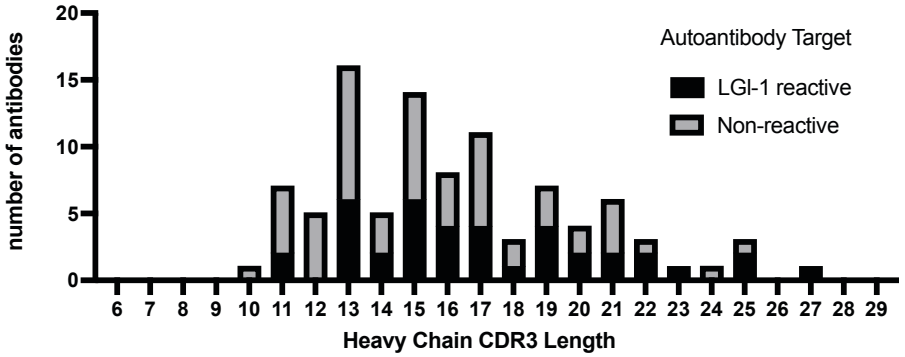

FIGURE 4. Comparison of LGI-1 reactive and non-reactive autoantibodies by CDR3 Length. Total Abs by CDR3 amino acid length are shown, with each subgroup, nonreactive (gray) and LGI-1 reactive (black), delineated.

DISCUSSION

Studying and defining the Ab repertoire in immune-mediated diseases can provide a platform for understanding their pathogenesis and present diagnostic and therapeutic opportunities to look for potential diagnostic markers and therapeutic targets. The current study provides an overview of autoimmune pediatric neurologic diseases, looking specifically at the characteristics of mAbs isolated from patients diagnosed with autoimmune neurologic diseases (e.g., anti-NMDAR encephalitis, limbic encephalitis). In our review of the available data, there was a striking paucity of specific Abs described for many of these conditions. Due to the commonality of the condition and availability of data, the main focus was describing the characteristics of NMDAR and LGI-1 autoantibodies in terms of CDR3 length, H chain family usage preference of V, D, and J gene segment, isotype use, and clonality.

CDR3 is the most diverse and important CDR in Ag recognition. The diversity is mainly generated by V(D)J recombination. An increase in the CDR3 length of the BCR is associated with Ab polyreactivity and autoimmunity (56). In our study, the mean CDR3 length of the total NMDA autoantibodies was 15.17 aa (SD 3.7), whereas the mean CDR3 length of the total LGI-1 autoantibodies was 16.22 aa (SD 3.76). The mean lengths of NMDA and LGI-1 autoantibodies were 14.74 aa (SD 3.43) and 17.17 aa (SD 3.95), respectively. Previous studies have shown that the lengths of human CDR3 are normally distributed (57, 58). In a diverse repertoire, the distribution of CDR3 lengths resembles a truncated or discretized Gaussian distribution (57). If the size distribution exhibits skewing, this can be an indication of an unexpected overabundance of clones undergoing abnormal development or perhaps reacting to a specific Ag (59). Using the D'Agostino–Pearson normality test, our data showed that NMDA autoantibody CDR3 lengths were normally distributed whereas LGI-1 autoantibody CDR3 length distribution was skewed. The variability seen in these data supports that there is not a global structural restriction on the types of Abs that can be neuro-autoantibodies.

Defining Ab isotypes is vital for the full description and analysis of the BCR repertoire. In anti-NMDAR encephalitis, Dalmau (60) reported that only IgG Abs are pathogenic, as they can cause the reduction of NMDAR at synaptic and nonsynaptic levels. Another study by Hara et al. (61) reported that serum or CSF IgG NMDAR-reactive Abs are highly specific for anti-NMDAR encephalitis whereas IgA or IgM Abs occur infrequently and nonspecifically in patients with or without autoimmune encephalitis (e.g., stroke, dementia), and Abs using these isotypes do not alter the receptor levels. IgG1 and IgG3 were reported as the main classes of pathogenic Abs against NR-1 whereas IgG4 was not previously reported (62, 63). Our analysis showed that IgG (IgG1, IgG2, and IgG3) was the main isotype detected and was the only isotype among the NR-1–reactive group, further supporting that pathogenic NMDAR-reactive autoantibodies mainly use IgG. IgG4 was not detected among the reactive group.

In contrast to anti-NMDAR encephalitis, LGI-1 encephalitis is known to be caused primarily by IgG4 Abs (64). A study by Gadoth et al. (65) confirmed the presence of IgG4 as the major

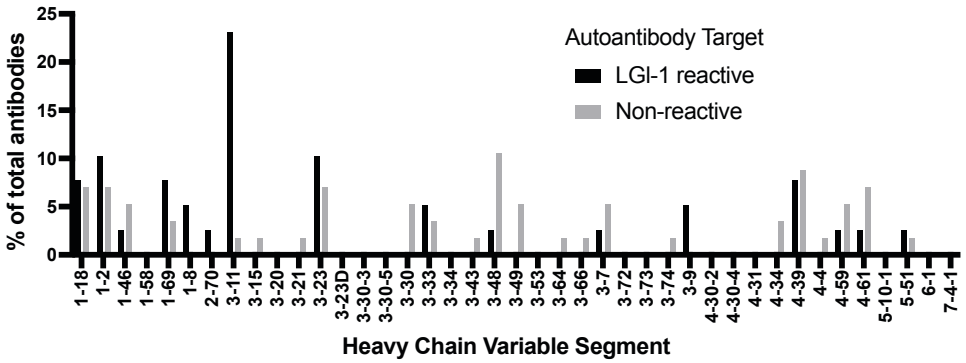

FIGURE 5. IGHV gene family use: LGI-1 reactive versus LGI-1 nonreactive. Percentages of total Abs utilizing the specific H chain variable segment genes are shown. Gene usage of LGI-1–reactive autoantibodies (black bars) and LGI-1 nonreactive autoantibodies (gray bars) paired comparisons for each gene segment are shown.

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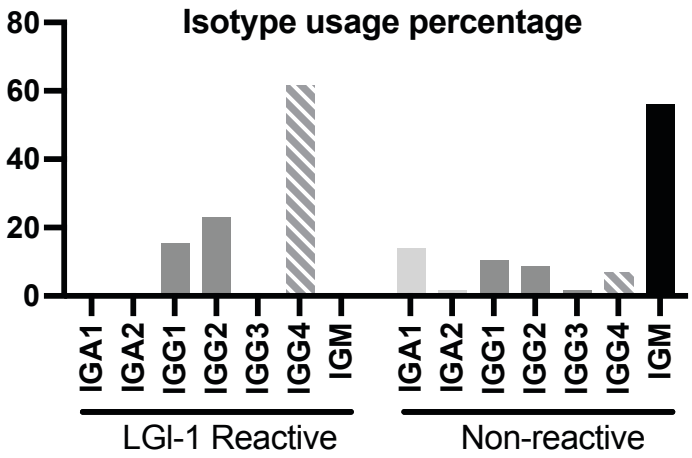

FIGURE 6. LGI-1 autoantibody isotypes.

IgG (dark gray) with IgG4 (striped), IgA (light gray), and IgM (black) are shown. IgG4 was the most abundant among the reactive group, whereas IgM was the most abundant among the nonreactive clones. Series 1, LGI-1 reactive; series 2, LGI-1 nonreactive.

isotype in patients with LGI-1 encephalitis and did not detect IgG2 or IgG3. Recently, it has been reported that the presence of high IgG4 titers in the CSF strongly correlates with worse outcome and that specific IgG isotypes may be predictive of refractory symptoms such as IgG1 and IgG4 (65, 66). Our analysis reviewed data from IgG1, IgG2, IgG3, and IgG4 subtypes. IgG4 represented one third the total and half of the IgG LGI-1 autoantibodies. IgG2 and IgG3 were part of the Ab repertoire, although IgG3 was not detected among the LGI-1–reactive clones. Our findings suggest that use of IgG4 may not be a requirement for all forms of LGI-1 encephalitis.

Due to the relative paucity of published data in this area, firm conclusions are difficult to form. From this review, there is not a specific set of characteristics of neuro-autoantibodies, as there appear to be no hard and fast restrictions on CDR length, isotype, variable segment, and mutations notable. However, between targeted Ags, there may be distinct restrictions. These differences likely are multifactorial, including initial development of such Abs with deregulation of negative selection and T cell help. Recent data support that HSV encephalitis (and other viral infections) can predispose to the development of NMDAR autoantibodies (67, 68). As viruses are complex Ags, the local development of initial B cell responses to a virus may allow a wide variety of clones that can cross-react to self-antigens.

Our analysis of published data shows that a diverse repertoire of NMDA autoantibodies exists, including NR-1–reactive and nonreactive clones. Development of neuroantibodies may be based on other methods, such as failure of negative selection. A mechanism such as deregulation of negative selection may better explain the findings with LGI-1, where Abs targeting other proteins are rare. The breadth of the response seen with NMDA autoantibodies, if seen in other conditions, may point to an inciting infectious incident, rather than a rare failure to negatively select unique autoreactive B cell clones.

The current study is not without limitations. Variations in methodology, sample sources, subjects, and analytical techniques across reviewed studies could have introduced bias and hinder direct comparisons. Although autoimmune neurologic conditions are common, there have been limited numbers of studies that describe the associated mechanisms, sequences, and other relevant data. Methodological variations across the available studies limited further statistical analysis. Overall, published data do not address mechanisms or specific epitope-targeting correlations to disease state. Our study does shine a light on the lack of data in this area with limited described Abs, relative lack of functional relativity, and lack of specific epitope correlation to pathophysiology. The field would benefit from further rigorous studies that describe such antibodies, publish these sequences, further confirm Ag targeting, and provide insight on clinical correlation.

DISCLOSURES

The authors have no financial conflicts of interest.

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