BRAIN-TARGETED AUTOIMMUNITY: BEYOND MULTIPLE SCLEROSIS

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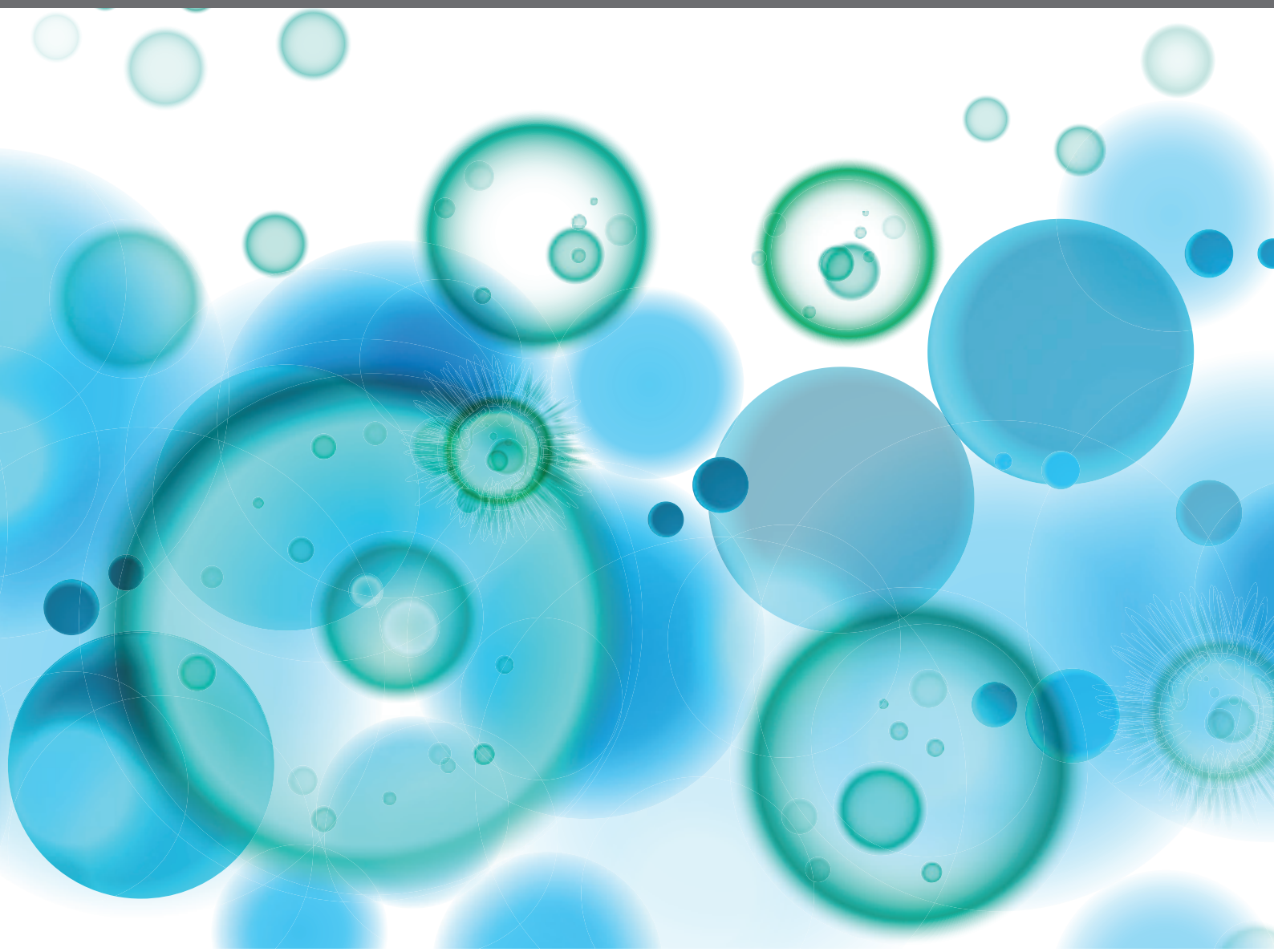

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BRAIN-TARGETED AUTOIMMUNITY: BEYOND MULTIPLE SCLEROSIS

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EDITORIAL
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Editorial: Brain-Targeted Autoimmunity: Beyond Multiple Sclerosis

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Keywords: autoimmunity, central nervous system, autoantibodies, T-cell, neurodegeneration

Editorial on the Research Topic

Brain-Targeted Autoimmunity: Beyond Multiple Sclerosis

INTRODUCTION

There is mounting evidence indicating that brain-targeted autoimmunity develops in a large range of neurological conditions in which the involvement of the adaptive immune system was not previously envisioned. Beyond multiple sclerosis and a few central nervous system (CNS) disorders acknowledged to be autoimmune by nature, it now appears that multiple neurological conditions are accompanied by brain-targeted autoimmune processes. The eleven articles gathered in this Research Topic fall into four main research themes, which represent the likely focus of major investigative efforts in the near future: i) links between physiological and pathological brain-targeted autoimmunity, ii) the functional impact of post-lesional brain-targeted autoimmunity, iii) the role of brain-targeted autoimmunity in neurodevelopmental and neurodegenerative disorders, iv) the emergence of new clinical entities associated with brain-targeted autoimmunity. In this editorial, we first provide a brief summary of the main advances presented by these articles. We then discuss the concept of "brain-targeted autoimmunity" and how to refine it in order to improve our pathophysiological understanding of a growing array of CNS diseases.

UNRAVELING THE LINKS BETWEEN PHYSIOLOGICAL AND PATHOLOGICAL BRAIN-TARGETED AUTOIMMUNITY

In their article, Arevalo-Martin et al. show that in patients suffering from spinal cord injury, elevated levels of autoantibodies directed against CNS antigens can be identified as early as 5 days post-injury. These autoantibodies were determined to be naturally-occurring following their detection in the serum of healthy individuals as well. Along this line, Chantran et al. propose an original

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pathophysiological scheme in which naturally-occurring autoantibodies directed against the amyloid beta peptide Aβ1-40 may be responsible for the development of cerebral amyloid angiopathy complications. Finally, the data mining article by Nataf et al. points to a potential role of B-cells in shaping physiological autoimmune responses against neurodegeneration-associated proteins such as amyloid beta, alpha-synuclein and tau.

POST-LESIONAL BRAIN-TARGETED AUTOIMMUNITY

Owens et al. demonstrate that in patients suffering from intractable pediatric epilepsy, irrespective of etiology, brain-infiltrating T-cells are present in resected brain tissue samples. Thus, in both patients with immune-mediated epilepsy (more specifically Rasmussen encephalitis) and patients with epilepsy of non-immune origin, a robust immune response involving myeloid cells and T-cells is observed in the brain parenchyma. In their article, Javidi and Magnus review and discuss previous experimental findings assessing the kinetics and functional role of adaptive immune responses during the course of stroke and brain injury.

BRAIN-TARGETED AUTOIMMUNITY IN NEURODEVELOPMENTAL AND NEURODEGENERATIVE DISORDERS

While Yshii et al. describe the pathophysiological mechanisms of paraneoplastic cerebellar degeneration and the resulting therapeutic implications that might be drawn from these experimental findings, Garretti et al. discuss recent data obtained in Parkinson's disease patients and mouse models regarding the occurrence of autoreactive T-cells targeting neoepitopes of alpha-synuclein. Finally, Gata-Garcia and Diamond review the experimental and epidemiological evidence supporting the idea that maternal brain-reactive autoantibodies might impact fetal brain development and contribute to long-term behavioral and/or cognitive phenotypes, including autism spectrum disorders.

EMERGENCE OF NEW CLINICAL ENTITIES ASSOCIATED WITH BRAIN-TARGETED AUTOIMMUNITY

Three articles of this special issue relate to clinical, imaging and/or biological findings that are directly relevant to the practice of neurology and the diagnosis, therapeutic approach, and management of patients with autoimmune CNS disorders. Such pathologies include autoimmune glial fibrillary acidic protein astrocytopathy (Shan et al.) and autoimmune encephalitis (Lv et al.; Nóbrega et al.).

CONCLUSION

Articles from this Research Topic highlight that the definition of "brain-targeted autoimmunity" requires further investigation and clarification. Obviously, demonstrating that autoantibodies and/or T-cells react against brain antigens under pathological conditions is not sufficient to formally conclude that such autoimmune processes play a significant pathophysiological role. This is particularly true when it comes to inferring that brain-targeted autoimmunity is a *primum movens*. Thus, animal models are critical to causally implicate such brain-targeted autoimmune processes in related disorders, and to decipher the underlying cellular and molecular mechanisms. With this framework, the paper by Yshii et al. offers a nice illustration of such experimental evidence in the context of paraneoplastic cerebellar degeneration. It becomes even more important to develop experimental approaches in animal models in light of new evidence that autoimmunity against CNS antigens also develops during the course of CNS disorders which are not primarily autoimmune, including prevalent and devastating conditions such as stroke and brain trauma (Javidi and Magnus), epilepsy (Owens et al.) and spinal cord injury (Arevalo-Martin et al.). Interestingly, at least in the context of stroke and spinal cord injury, CNS-targeted adaptive immune responses are found to develop early in the course of CNS injury and to likely stem from naturally-occurring autoantibodies and pre-existing self-reactive T cells. Such findings suggest that, while exerting physiological functions in the steady state, naturally-occurring adaptive immune responses might induce disease-specific effects which are somehow inhibited under normal conditions. In line with this view, the paper by Chantran et al. put forward an interesting pathophysiological scheme in which naturally-occurring autoantibodies directed against amyloid beta are proposed to serve protective functions during aging but can also contribute to complications of cerebral amyloid angiopathy in some individuals. Although the mechanisms underlying the development of naturally-occurring brain-targeted autoimmunity are still uncertain, the data mining analyses reported by Nataf et al. indicate that human B-cells physiologically express a large range of proteins involved in neurodegenerative processes and that these cells might be involved in the antigenic presentation of peptides derived from such proteins. On this basis, the authors posit that a main function of physiological brain-targeted autoimmunity is to prevent the accumulation of aggregated forms of amyloid beta, alpha-synuclein and/or tau proteins in the periphery. Although this assertion seems contradictory to the evidence of pathogenic autoimmune responses in patients with neurodegenerative conditions, Garretti et al. illustrate that pathogenic autoimmune T-cell responses against alpha-synuclein are indeed directed against neoepitopes, which occur under specific inflammatory conditions. Therefore, it is conceivable that protective and deleterious autoimmune responses to alpha-synuclein are directed against distinct epitopes and may actually co-exist in the same individual. This point is of particular importance as it suggests that harnessing the adaptive immune system for treating neurodegenerative

disorders might require epitope-specific manipulations in order to achieve effectiveness and safety.

AUTHOR CONTRIBUTIONS

SN wrote the paper. RL, SH and GD provided corrections. All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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Elevated Autoantibodies in Subacute Human Spinal Cord Injury Are Naturally Occurring Antibodies

Angel Arevalo-Martin[1], Lukas Grassner[2,3,4], Daniel Garcia-Ovejero[1], Beatriz Paniagua-Torija[1], Gemma Barroso-Garcia[5], Alba G. Arandilla[5], Orpheus Mach[2], Angela Turrero[1], Eduardo Vargas[6], Monica Alcobendas[6], Carmen Rosell[7], Maria A. Alcaraz[6], Silvia Ceruelo[6], Rosa Casado[6], Francisco Talavera[6], Ramiro Palazón[6], Nuria Sanchez-Blanco[8], Doris Maier[2], Ana Esclarin[6] and Eduardo Molina-Holgado[1]*

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Spinal cord injury (SCI) results in long-term neurological and systemic consequences, including antibody-mediated autoimmunity, which has been related to impaired functional recovery. Here we show that autoantibodies that increase at the subacute phase of human SCI, 1 month after lesion, are already present in healthy subjects and directed against non-native proteins rarely present in the normal spinal cord. The increase of these autoantibodies is a fast phenomenon–their levels are already elevated before 5 days after lesion–characteristic of secondary immune responses, further supporting their origin as natural antibodies. By proteomics studies we have identified that the increased autoantibodies are directed against 16 different nervous system and systemic self-antigens related to changes known to occur after SCI, including alterations in neural cell cytoskeleton, metabolism and bone remodeling. Overall, in the context of previous studies, our results offer an explanation to why autoimmunity develops after SCI and identify novel targets involved in SCI pathology that warrant further investigation.

Keywords: spinal cord injury, neurotrauma, natural autoantibodies, proteomics, autoimmunity

INTRODUCTION

Spinal cord injury (SCI) results in neurological and systemic alterations that dramatically interfere with patient quality of life. Several factors contributing to SCI pathology have been proposed in an attempt to identify novel targets for therapeutic intervention. One of these are autoantibodies (AAb) against central nervous system antigens, which are elevated in SCI patients and are pathogenic in rodents (1–5). In mice, SCI induces B cells to proliferate and differentiate into immunoglobulin (Ig) G secreting B cells, which results in increased levels of IgG in peripheral blood (3). Injecting these circulating IgG into the spinal cord of intact animals induces necrosis, inflammation and motor impairment (5). Accordingly, mice genetically devoid of B cells, which do not produce antibodies, develop smaller lesions and recover better from SCI. Although the

body of evidence suggest that suppressing antibody production could be an effective therapeutic strategy to promote recovery, paradoxically, SCI patients also develop a well-defined immune depression syndrome that increases their susceptibility to infections, the major cause of morbidity and mortality after SCI (6, 7). Like in humans, mice show impaired immune responses after SCI –including decreased antibody production– that increase their susceptibility to infections (8–10). Consequently, therapies directed to interfere with antibody production and/or their effector mechanisms should not be based on a general immunosuppression, but specifically directed toward those responses related to SCI pathology. So far, significant increased levels of AAb have been described against myelin basic protein (MBP), ganglioside GM1 (GM1), myelin galactocerebroside (GalC), glial fibrillary acidic protein (GFAP) and collapsing response mediator protein-2 (CRMP-2) in humans after SCI (1, 2, 4, 11–13). Also, by incubating patient blood sera with a phage display of a human spinal cord cDNA expression library, a set of five AAbs against 26S proteasome non-ATPase regulatory subunit 4 (PSMD4), glyceraldehyde-3-phosphate dehydrogenase (G3P), myeloma-overexpressed gene protein (MYEOV2), protein S100-B (S100B) and adipocyte enhancer-binding protein 1 (AEBP1) has been proposed to be enriched in patients, although individually none of these AAbs reached statistical significance (14). However, the complete repertoire of AAb specificities induced after SCI remains unknown because most studies have been done by selecting the antigenic determinants to be tested "*a priori*," not by unguided discovery methods.

Why immune depression and autoimmunity co-occur after SCI remains unsolved. A proposed explanation is that autoimmunity is inversely related to the magnitude of immune depression (6). Immune depression after SCI is more pronounced after cervical and high thoracic lesions (injuries above the 5th thoracic spinal segment) due to the dysregulation of the sympathetic nervous system below the lesion site (10, 15). Accordingly, it has been reported that high thoracic lesions, but not low thoracic, inhibit the production of antibodies after immunization with exogenous antigens in mice (8, 15). However, cervical injuries increase AAb levels against spinal cord self-antigens (16). These opposed results between the responses of antibodies against exogenous and self-antigens may be explained by the observations in rodent experimental models showing that (i) SCI only impairs the generation of new antibody responses but preserves already existing immunity (17) and that (ii) AAbs detected after SCI may exist before lesion (18). Therefore, AAbs observed after SCI could be the result of secondary immune responses of previously existing immunity; i.e., these AAbs could be naturally occurring AAbs. These are normally present in healthy subjects as IgMs and IgGs (19–24) and have been suggested to be either involved in homeostatic and healing processes by recognizing proteins with modifications induced by cell stress or damage (25–27) as well as being the origin of pathological AAbs (25, 28–30).

By using a hypothesis-free methodology we show that AAbs against damaged spinal cord are already detectable in the sera of normal subjects and that AAbs against 16 different targets are significantly increased in the subacute phase after SCI. Among these AAb targets, 13 are reported for the first time to be increased after SCI and unveil a portrait of cellular and molecular alterations with therapeutic interest for SCI.

MATERIALS AND METHODS

Patients and Healthy Control Subjects

Fifty-two patients with traumatic SCI and ten patients with traumatic central cord syndrome were recruited at Hospital Nacional de Paraplejicos (Toledo, Spain)—a national center specialized in spinal cord injury—and at Trauma Centre Murnau (Bavaria, Germany)—a cross-regional trauma center with a specialized spinal cord injury department. All patients included in this study were examined at a median time of 31 ± 1 days after injury, and a subgroup of eleven patients from the fifty-two SCI individuals were additionally examined at a mean time of 2 ± 0.4 days after injury (range 0–5 days). All patients fulfilled the inclusion and exclusion criteria and gave their informed consent to participate. Inclusion criteria were:

- Males and females
- At least 18 years old
- Any neurological level of injury, but cauda equine syndrome
- Complete and incomplete lesions
- If patient was treated with glucocorticoids, it should have passed at least 7 days after ending the treatment

Exclusion criteria were:

- Diagnosed autoimmune disorder
- Diagnosed tumor
- Diagnosed neurodegenerative disease

Sensorimotor function of patients was evaluated following the International Standards for Neurological Classification of Spinal Cord Injury scale (ISNCSCI). All evaluations were performed by trained personnel with experience certified by the centralized ISNCSCI training course at Heidelberg University Hospital. Lesion level and severity of patients is summarized in **Table 1**.

Age and gender-matched voluntary healthy individuals were recruited by the Department of Occupational Health at Hospital Nacional de Paraplejicos (**Table 2**).

The study protocol and the informed consent sheet were evaluated and approved by the Ethics Committee of the Toledo Health Care Area and by the Ethics Committee of the Bavarian Medical Board. The study follows and adheres to the World Medical Association Declaration of Helsinki and is registered at the public database Clinicaltrial.gov (registration number NCT02493543).

Blood Sera

Peripheral blood was collected in anti-coagulant free tubes by venepuncture at the medial cubital vein. Blood clot was allowed to form by maintaining the tubes for 45 min at room temperature (RT) and 1 h at 4°C. Blood was centrifuged at 1,500 g for 20 min at 4°C and serum was collected, aliquoted and stored at −80°C until used.

TABLE 1 | Lesion level and severity of patients.

NLI[a]	AIS A (#)	AIS B (#)	AIS C (#)	AIS D (#)
Cervical	14	1	6	1
Thoracic	23	2	1	0
Lumbar	2	1	1	0

[a]*NLI, neurological level of injury.*

TABLE 2 | Demographical characteristics of patients and control subjects.

	n	Age, years (mean ± sem)
SCI[a] total	52	40.6 ± 2.2
Men	46	39.1 ± 2.3
Women	6	52.3 ± 8.1
CTL[b] total	16	45 ± 2.8
Men	13	45.0 ± 3.2
Women	3	47.7 ± 6.7

[a]*SCI, spinal cord injury patients.*
[b]*CTL, control healthy subjects.*

Whenever a blood sample was taken, a standard hematological analysis including red and white cells count and related parameters determination was performed by the clinical laboratories at Hospital Nacional de Paraplejicos (Toledo, Spain) or at Murnau Trauma Centre (Murnau, Germany).

Animals and Experimental Spinal Cord Injury (SCI)

Young adult male Wistar rats (295–315 g, 12 weeks of age) were obtained from Harlan-Interfauna Iberica (Barcelona, Spain) and were maintained in our animal facilities on a 12:12-h light:dark cycle, receiving food and water *ad libitum*. All experimental procedures were approved by our institutional animal use and care committee. Rats were handled in accordance with the guidelines published by Spain and the European Union (RD1201/2005, 2010/63/EU).

SCI at T8 vertebral level was induced as previously described (31). Briefly, we anesthetized rats with an intraperitoneal injection of sodium pentobarbital (45 mg/Kg, Normon Veterinary Division, Madrid, Spain) and Xylacine (10 mg/Kg, Calier, Barcelona, Spain). After confirming the absence of reflexes, we injected atropine (50 μg/Kg, Brown Medical, Barcelona, Spain) and we applied artificial tears to prevent corneal abrasion and infection. We performed a laminectomy of T8 vertebra and we stabilized the vertebral column by clamping spiny processes of T7 and T9 vertebras. Spinal cord contusion/short compression was performed with the "Infinite Horizon™" device (Precision Systems and Instrumentation, Lexington, KY, USA), applying a force of 200 Kdyn and a compression time of 5 seconds over the exposed cord. Force and displacement curves generated by the impactor were checked to confirm that injuries were done with similar values and profiles without artifacts indicative of an erroneous/abnormal lesion.

Post-operative care included analgesia (Buprenorphine), prophylactic antibiotic treatment (Enrofloxacine) and hydration (saline serum) during the first week after injury. Bladder was manually voided until animals were sacrificed.

We minimized the number of rats subjected to SCI. In detail, three rats were lesioned to detect endogenous IgGs in the spinal cord at 1 day after injury by immunohistochemistry (results shown in **Figure 5**); two to be employed in immunohistochemistry with human sera at 14 days after injury (results shown in **Figures 1–3**); two to isolate spinal cord proteins at 1 day after injury to be employed in Western blot experiments with human sera (results shown in **Figure 1**); and two to isolate spinal cord proteins at 28 days after injury to be used in Western blot experiments with human sera (results shown in **Figure 1**). In addition, two intact rats were sacrificed and perfused for immunohistochemistry experiments (results shown in **Figures 1–3**, **5**) and two other intact rats were sacrificed to isolate spinal cord proteins for Western blot experiments (**Figure 1**).

Processing of Rat Spinal Cord for Immunohistochemistry

At 1 or 14 days after injury, rats were anesthetized with an intraperitoneal injection of sodium pentobarbital and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. The spinal cord was dissected out and post-fixed for 4 h in the same solution at 4°C. Tissue blocks of 1 cm were embedded in low-melting agarose and cut into 40 μm thick coronal sections with a Leica VT1000S vibratome (Madrid, Spain). Sections were stored in Olmos solution until they were used for free-floating immunohistochemistry.

Inmunohistochemistry

Rat spinal cord was processed as previously described (31) using as primary antibodies the IgGs contained in sera samples or commercial antibodies against MBP (1:1,000, Covance, Princeton, NJ, USA), NFM (1:500, Aves Lab, Tigard, Oregon, USA), GFAP (1:2,000, Dako, Glostrup, Denmark) and APC (1:300, Calbiochem, Darmstedt, Germany). To detect these antibodies we used Cy3 conjugated anti-human IgG (1:1,000, Jackson Immunoresearch, Ely, UK), Cy5 anti-chicken IgY, Alexa Fluor-594 anti-rabbit IgG (1:1,000, Invitrogen, Barcelona, Spain) and Alexa Fluor-488 anti-mouse IgG (1:1,000, Invitrogen). To detect endogenous IgG in rat spinal cord, HRP-conjugated anti-rat IgG (1:1,000; Jackson Immunoresearch) was used and their localization was detected by DAB-peroxidase reaction. Also, HRP-conjugated anti-rabbit IgG (1:1,000; Jackson Immunoresearch) was employed as a control of specificity. When performed, nuclei were counterstained with Hoescht 33,258 (1:5,000; Invitrogen).

Samples were analyzed with a LEICA SP5 confocal microscope. All post-capture image modifications were identically performed between groups, including cropping, noise reduction and minor adjustments to optimize contrast and brightness.

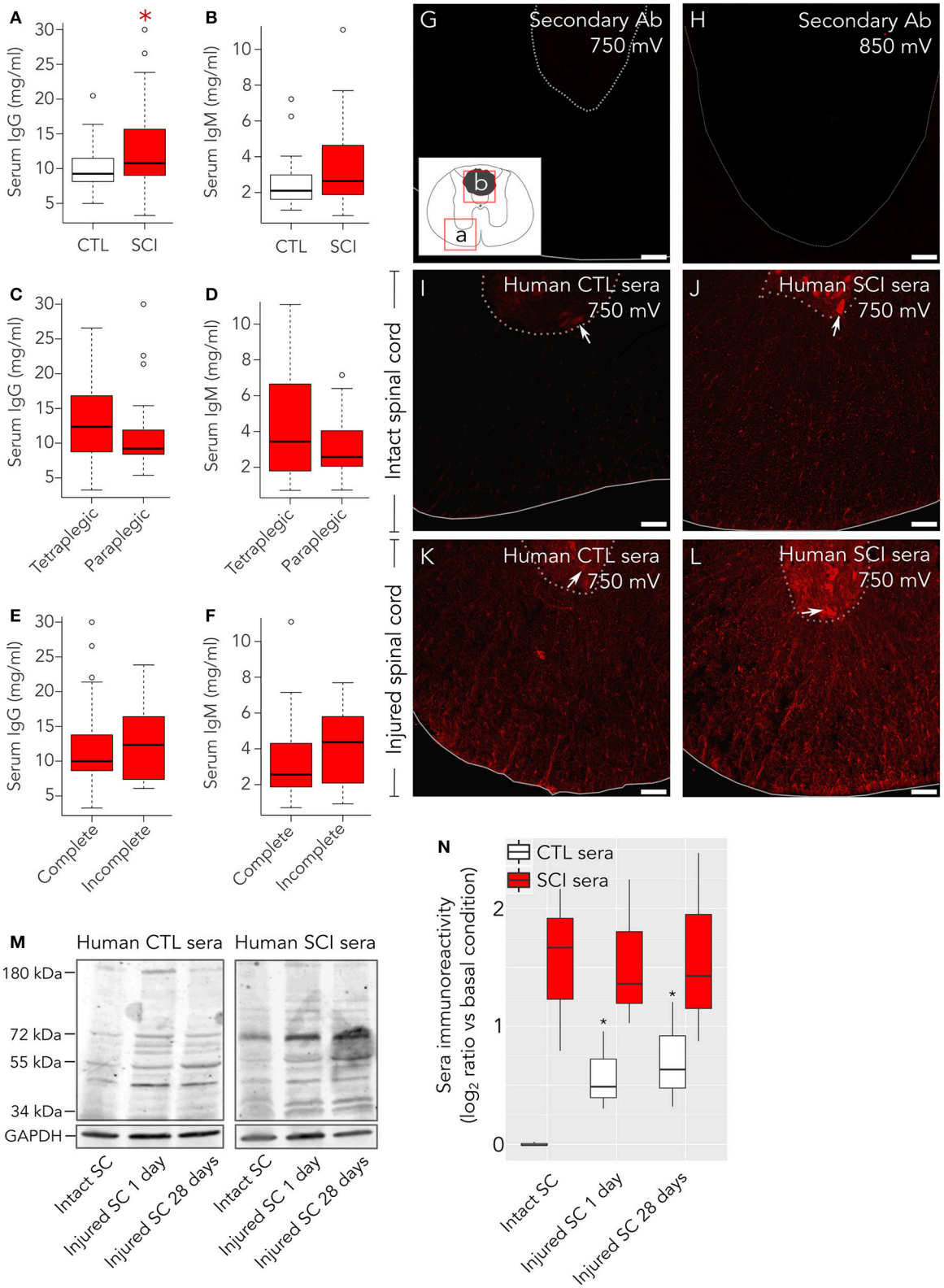

FIGURE 1 | Healthy control subjects present autoantibodies preferentially directed against damaged spinal cord. As measured by ELISA, **(A)** serum IgG concentration is increased in patients compared to control healthy individuals, while **(B)** serum IgM levels are not statistically different between both groups. Neurological level

(Continued)

FIGURE 1 | of lesion **(C,D)** and severity (AIS **A** complete vs. sensory incomplete; **E,F**) do not significantly affect the levels of IgGs nor IgMs in patients. **(G–L)** Immunohistochemistry was performed to detect binding of serum IgGs to intact and injured rat spinal cord. All experiments were performed in parallel, to avoid potential batch effects. Confocal microscopy images (single confocal plane) were acquired by setting up photomultiplier voltage (sensitivity) to values far beyond those where unspecific background may arise. **(G)** With confocal microscope photomultiplier at 750 mV no signal is observed on injured spinal cord when only secondary anti-human IgG is added. **(H)** Even at higher intensities, 850 mV, in the lesion border, where autofluorescence is more intense, no signal is detected in these experiments. Rectangles **(a,b)** in the insert of panel **(G)** indicate the spinal cord regions shown in panels **(G,I–L) (a)** or in panel H **(b)**. **(I)** Serum IgGs form control individuals weakly react with intact rat spinal cord, preferentially binding to motoneurons in the ventral horn (arrow), but strongly react with glial and neuronal cellular profiles in injured rat spinal cord **(K)**. Serum IgGs from SCI individuals react with intact tissue **(J)** and with injured spinal cord **(L)**. **(M)** Representative Western blots showing reactivity of serum IgGs from control individuals (CTL, left panel) or spinal cord injury patients (SCI, right panel) over protein extracts from intact rat spinal cord (SC), injured rat SC at 1 days after lesion or injured rat SC at 28 days after injury. **(N)** Quantification of overall IgG immunoreactivity in Western blots show that CTL serum IgGs ($n = 3$) present a significant higher immunoreactivity against proteins isolated from injured rat spinal cord than against proteins isolated from intact rat spinal cord. On the other hand, SCI sera IgGs ($n = 3$) show higher immunoreactivity than control serum IgGs at any condition (2-way ANOVA $p = 0.006$). Basal condition is defined as the mean value of CTL serum IgG immunoreactivity against proteins isolated from intact rat spinal cord. Gray line in **(G,I–L)** marks spinal cord border, and pointed-line delimits the ventral horn. Gray line in **H** depicts lesion border. Arrows in **C–F** point to ventral horn motoneurons. $^*p < 0.05$. Scale bar, 75 μm.

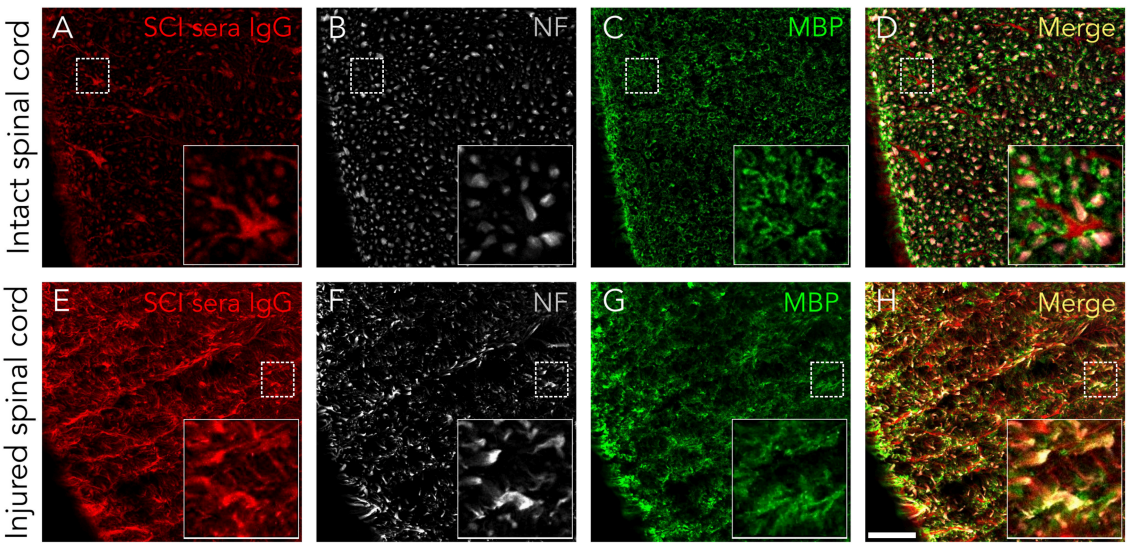

FIGURE 2 | Serum IgGs react with antigens expressed on axons and disorganized myelin. IgGs from SCI individuals (in red) bind to normal appearing axons (NF-positive, in white) in control rat spinal cords **(A–D)** as well as to dystrophic axons in injured tissue **(E–H)**. However, IgGs do not seem to react with normally appearing myelin (as detected by being MBP-positive, in green), although they can be localized in some disorganized myelin structures in SCI tissue (yellow signal in **G, H**). All images are a single confocal plane. NF, neurofilament. MBP, myelin basic protein. Scale bar, 50 μm.

IgG and IgM Enzyme-Linked Immunosorbent Assays (ELISA)

Total levels of serum IgG and IgM were determined with Human IgG or IgM Platinum ELISA kits (eBioscience-Thermo Fisher Scientific, Madrid, Spain) following manufacturer's instructions. All samples were measured by duplicate and those with a coefficient of variation >10% were repeated. Dilution curves of standard IgG or IgM were performed for each 96-well plate. Consistency of results among different plates was confirmed by introducing the same sample from a healthy control subject in all plates and observing reproducible values. When the values of this sample were not reproducible, the whole plate was discarded and repeated.

Protein Isolation From Human and Rat Spinal Cord

Protein isolation was performed with ReadyPrep™ protein extraction kit for soluble and insoluble proteins (Bio-Rad, Madrid, Spain). Briefly, tissue was mechanically homogenized and sonicated in chilled 40 mM Tris base buffer supplemented with protease inhibitors (Complete Mini, Roche Diagnostics, Mannheim, Germany). The volume of buffer employed for every tissue sample was escalated to be in the range of 200 mg of tissue per 1 ml of buffer. Samples were centrifuged at 15.000 × g for 30 min at 4°C and supernatants –fraction containing hydrophilic proteins– were collected. Pellets were resuspended and sonicated in the same volume of 2-D rehydration/sample buffer, containing 7 M urea, 2 M thiourea, 1% ASB-14 detergent, 40 mMTris base and 0.001% bromophenol blue supplemented with 0.2% tributylphosphine (TBP) reducing agent. Samples were centrifuged at 15,000 g for 20 min at RT and supernatants –fraction containing hydrophobic proteins– were collected. Protein concentration of both insoluble and soluble fractions was determined by RC DC protein assay (Bio-Rad). Samples were aliquoted and stored at −80°C until further use.

In the case of rat spinal cord, intact and injured rats (at 1 and 28 days after lesion) were anesthetized with an

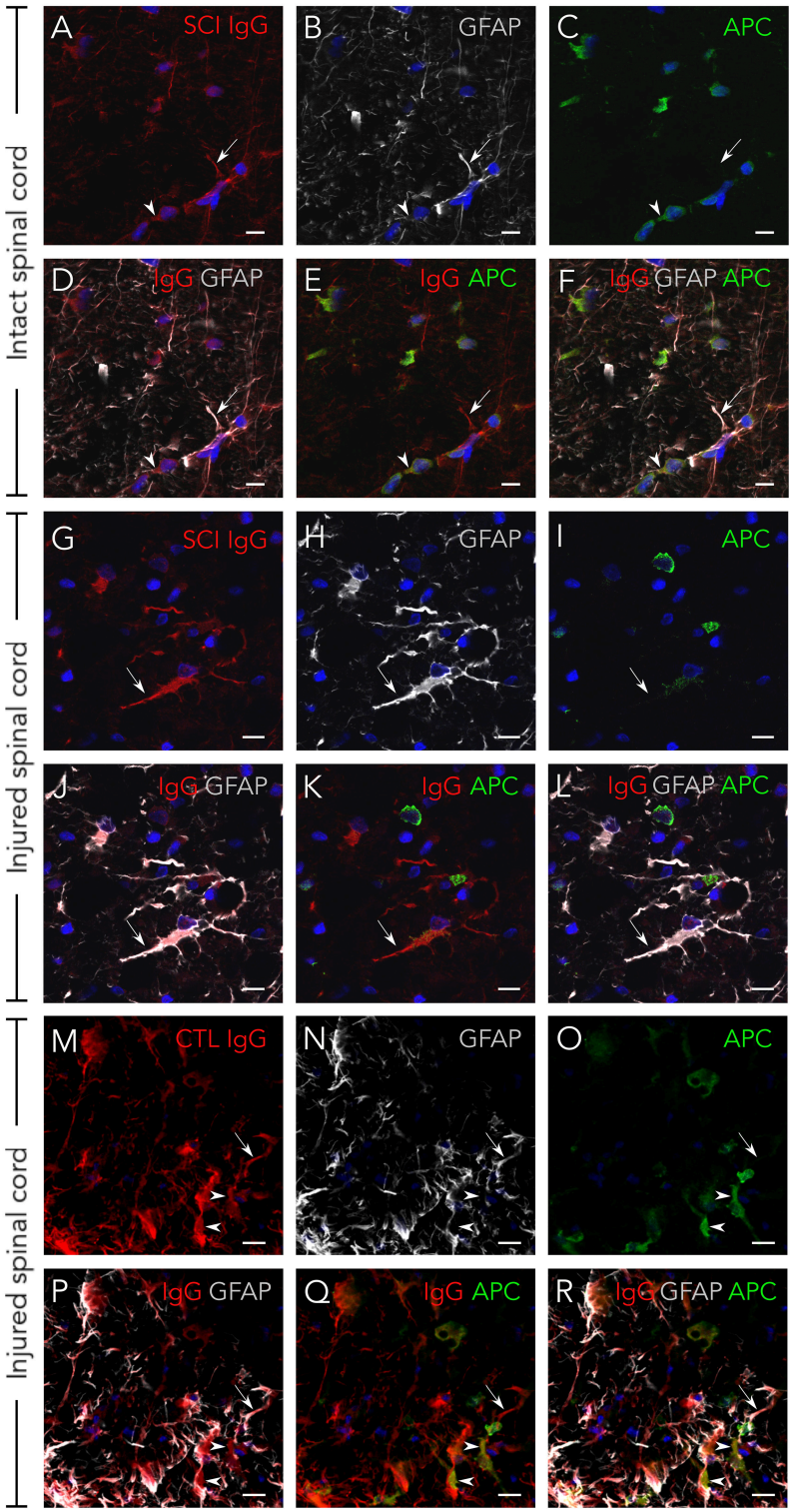

FIGURE 3 | Serum IgGs react with antigens expressed on astrocytes and oligodendrocytes. IgG from SCI individuals (in red) bind to astrocytes (GFAP-positive, in white) and oligodendrocytes (APC-positive, in green) both on intact spinal cord tissue **(A–F)** and on injured tissue **(G–L)**. The same immunostaining pattern is observed with IgGs from healthy individuals on injured tissue **(M–R)**. As shown in the micrographs, not all astrocytes or oligodendrocytes in the rat spinal cord are targeted by human IgGs. All images are maximal projections obtained from a z-stack formed by five confocal planes. GFAP, glial fibrillar acidic protein. APC, adenomatous polyposis coli. Arrows point to IgGs reacting on astrocytes and arrowheads to IgGs reacting on oligodendrocytes. Scale bar, 10 μm for all images.

intraperitoneal injection of sodium pentobarbital. The spinal cords were dissected out at 4°C and tissue blocks of 5 mm comprising the rostral part of the lesion epicenter were processed for protein isolation. Protein isolation and determination of their concentration was performed as described above, and samples were aliquoted and stored at −80°C until further use.

Western Blot With Rat Spinal Cord Tissue

Protein lysates were mixed with 5× Laemmli sample buffer and heated at 60°C for 5 min. Equal amounts of protein were resolved in 4–15% gradient Mini-PROTEAN® TGX™ precast protein gels (Bio-Rad) and electroblotted onto nitrocellulose membranes. Membranes were blocked for 30 min at room temperature with synthetic blocking solution (Invitrogen, Madrid, Spain) and incubated overnight at 4°C with a pool of human sera (1:500 dilution) from control or SCI individuals. Pools were constructed by mixing equal volumes of sera from four different subjects. Three different serum pools of intact individuals and three different pools of SCI patients were used. Membranes were then incubated at room temperature for 90 min with Alexa Fluor 790-conjugated anti-human IgG antibodies (Fc_γ fragment specific, 1:1,000; Jackson Immunoresearch). Protein loading was evaluated by incubating the membranes overnight at 4°C with anti-GAPDH antibody (1:500,000; Abcam, Cambridge, UK) followed by incubation with secondary Alexa-680 anti-mouse IgG (1:5,000; LI-COR Biosciences) for 90 min at room temperature. Blots were visualized and acquired using Odyssey® CLx equipment (LI-COR Biosciences, Lincoln, NE, USA).

Immunoreactivity of human serum IgGs against spinal cord proteins from intact or injured rats was quantified by determining the integrated intensity of the protein bands observed in each western blot (WB) lane using the FIJI distribution of ImageJ software (32). The sum of integrated intensities for a given lane was normalized to the protein loading in that lane estimated by measuring GAPDH integrated intensity.

Detection of Human Spinal Cord Targets of Autoantibodies by Two Dimensional-Western Blot

Due to the lack of non-fixed spinal cord samples from SCI patients and considering that autoantibodies could be directed against proteins newly expressed or modified by lesion, we searched for human spinal cord samples where inflammation- or cell death-induced modifications could be present. Therefore, we pooled proteins extracted from pathological spinal cord tissue obtained from Balo concentric sclerosis lesions, multiple sclerosis demyelinated areas and amyotrophic lateral sclerosis. In addition, since it could not be discarded that some autoantibodies might be directed against proteins more abundantly or exclusively present in control subjects, we also extracted proteins from non-pathological human spinal cord samples and mixed them in a final pool with pathological spinal cord tissue samples (**Supplementary Table 1**). Frozen non-fixed human spinal cord tissue blocks were kindly supplied by the Neurological Tissue Biobank of Hospital Clinic-IDIBAPS (Barcelona, Spain) and CIEN Foundation Tissue Bank (BT-CIEN; Madrid, Spain). Protein isolation was performed as described above. Proteins were precipitated using the 2D Clean Up Kit (Sigma, Madrid, Spain) following manufacturer's instructions and resuspended in labeling buffer containing 7 M urea, 2 M thiourea, 4% CHAPS and 30 mM Tris. After checking samples to be between pH 8.0–9.0, soluble and insoluble fractions were mixed in a 1:1 ratio. Proteins were then labeled with Cyanine 2 NHS ester minimal dye (Lumiprobe, Hannover, Germany) following manufacturer's instructions, for 30 min in dark conditions.

Non-lineal 11 cm strips, pH 3–10 (GE Healthcare, Little Chalfont, UK), were placed in a re-swelling tray (GE Healthcare) to actively rehydrate overnight with 150 μg Cy2-labeled proteins. Isoelectric focusing was then performed in a Protean IEF cell unit (Bio-Rad), at 20°C, according to the following program: 30 min at 500 V, 1 h at 1,000 V (ramping), 3 h at 5,000 V (ramping) up to a total of 35,000 V/h. After isoelectrofocusing, strips were equilibrated in 1.5 M Tris, ph 8.8 buffer containing 6 M urea, 30% glycerol, 2% SDS and bromophenol blue, with the addition of 1% DTT for 20 min. Then, strips were incubated for 20 more min in the same buffer in the presence of 2.5% iodoacetamide. Proteins were then separated accordingly to their molecular weight in Criterion™ TGX™ AnykD™ midi precast gels (Bio-Rad).

After 2D-electrophoresis, proteins were transferred to low fluorescence PVDF membranes with a semi-dry protocol using Trans-Blot® Turbo™ transfer system (Bio-Rad). Membranes were blocked with BlockAid solution (Thermo Fisher Scientific, Madrid, Spain) for 30 min at RT and incubated overnight at 4°C with sera diluted 1:500 in PBS, 0.1% Triton X-100 and 10% BlockAid Solution. After several washing steps, membranes were incubated for 2 h at RT with secondary Cy3-conjugated goat anti-human IgG (Fc_γ fragment specific; 1:1,000; Jackson Immunoresearch) and Alexa-647 goat anti-human IgM ($Fc_{5\mu}$ fragment specific; 1:1,000; Jackson Immunoresearch).

We performed the complete procedure described above for every serum sample analyzed (no stripping and reblotting of membranes was performed). Reproducibility of the procedure was tested by randomly repeating five serum samples and confirming in a selected subset of protein spots that bound AAb values were similar between duplicates.

2D-WB Images Acquisition

2D-WB were visualized with the laser confocal scanner Typhoon™ Trio (GE Healthcare) and images were acquired at 50 μm/pixel with a 16-bit depth. Voltage of photomultipliers was maintained throughout all experiments. To determine voltage values for Cy2 channel –the whole spinal cord proteome– we confirmed that no signal was observed in a membrane without Cy2-labeled protein and we fixed a value that did not result in a saturated signal. For Cy3 and Alexa-647 channels we confirmed that no signal was detected when omitting the secondary antibodies and we fixed values that did not result in saturation.

2D-WB Analysis

Protein spots resulting from 2D-WB were segmented and matched among membranes with DeCyder 7.0 software (GE Healthcare). A careful visual inspection of segmentation and

matching was performed and errors were manually corrected. Also, spots containing technical artifacts, like air bubbles or fluorochrome precipitates, were eliminated. After background correction, the volumes (integrated densities) of every spot on Cy2, Cy3 and Alexa-647 channels were exported and processed with R programming language (33). Minimal labeling with Cy2 dye ensures labeling of a single lysine per protein molecule, so Cy2 integrated density values are proportional to protein loading. Cy3 integrated density –bound IgG AAb– and Alexa-647 integrated density –bound IgM AAb– are a function of their abundance and of that of their antigenic target. Therefore, every Cy3 and Alexa-647 values were corrected by the Cy2 value of their respective target. All values were transformed to $\log_2$ scale, so all IgG and IgM level values are the log ratio of their abundance relative to their antigenic target abundance:

$$IgG\ AAb = \log_2\left(\frac{Cy3\ integrated\ density}{Cy2\ integrated\ density}\right)$$

$$IgM\ AAb = \log_2\left(\frac{Alexa\_647\ integrated\ density}{Cy2\ integrated\ density}\right)$$

Comparison of AAb Binding to Their Targets Between SCI and Control Individuals

In every 2D-WB performed, any spinal cord protein recognized by IgG and/or IgM was annotated. This procedure was performed with all control and SCI individuals, generating a list of all antigenic targets visually detectable by AAbs in our population. Binding of AAb to their targets were compared between SCI and control subjects by two-tailed *t*-test. Multiple comparison correction was performed by Benjamini-Yekutieli method (34). All statistical analysis were performed in RStudio (35).

Identification of Antigenic Targets by MALDI/MS-MS

Targets of AAbs were in-gel digested using the Ettan Digester (GE Healthcare). The digestion protocol used was that of Shevchenko et al. (36) with minor variations: gel plugs were submitted to reduction with 10 mM dithiothreitol (Sigma Aldrich) in 50 mM ammonium bicarbonate (99% purity; Scharlau) and alkylation with 55 mM iodoacetamide (Sigma Aldrich) in 50 mM ammonium bicarbonate. The gel pieces were then rinsed with 50 mM ammonium bicarbonate in 50% Methanol (gradient, HPLC grade, Scharlau) and acetonitrile (gradient, HPLC grade, Scharlau) and dried in a Speedvac. Modified porcine trypsin (sequencing grade; Promega, Madison, WI, USA) at a final concentration of 20 ng/μl in 20 mM ammonium bicarbonate was added to the dry gel pieces and the digestion proceeded at 37°C overnight. Finally, 60% aqueous acetonitrile and 0.1% trifluoroacetic acid (99.5% purity; Sigma Aldrich) were added for peptide extraction. To confirm protein identity, digestion of every spot was repeated at least from two different gels. Then, proteins were identified by MALDI/MS-MS: 0.5 μl of each digestion solution were deposited using the thin layer method, onto a 384 Opti-TOF 123 × 81 mm MALDI plate (Applied Biosystems, Madrid, Spain) and allowed to dry at room temperature. The same volume of matrix (3 mg/mL α-cyano-4-hydroxycinnamic acid –Sigma Aldrich– in 60% acetonitrile, 0.1% trifluoroacetic acid) was applied on every sample in the MALDI plate. Samples were deposited by duplicate in every MALDI plate to avoid cross-contamination. MALDI-MS/MS data were obtained in an automated analysis loop using a 4800 Plus MALDI TOF/TOF Analyzer (Applied Biosystems). Spectra were acquired in the reflector positive-ion mode with a Nd:YAG, 355 nm wavelength laser, at 200 Hz laser frequency, and 1,000–2,000 individual spectra were averaged. The experiments were acquired uniform with fixed laser intensity. For MS/MS 1 kV analysis mode, precursors were accelerated to 8 kV in source 1, selected with a relative resolution of 200 (FWHM) and metastable suppression. Fragment ions generated by collision with air in a CID chamber were further accelerated by 15 kV in source 2. Automated analysis of mass data was performed using the 4000 Series Explorer Software version 3.7.0 (Applied Biosystems). Internal calibration of MALDI-TOF mass spectra was performed using two trypsin autolysis ions with m/z = 842.510 and m/z = 2,211.105. For MALDI-MS/MS, calibrations were performed with fragment ion spectra obtained for Glub-fibrinopeptide (4700 Cal Mix, Applied Biosystems). MALDI-MS and MS/MS data were combined through the ProteinPilot Version 5.0.1 to search a nonredundant protein database (Swissprot 2017_02) using the Mascot software version 2.5 (Matrix Science) with 50 ppm precursor tolerance, 0.6 Da MS/MS fragment tolerance, CAM (carbamidomethylcystein) as fixed modification, oxidized methionine as variable modification and allowing 1 missed cleavage. MALDI-MS/MS spectra and database search results were manually inspected in detail using the aforementioned software.

Maps of Protein Isoforms Targeted by AAbs

The observed molecular weight (MW) and isoelectric point (pI) of the proteins targeted by AAbs were calculated with the Biological Variation Analysis module implemented in DeCyder 7.0 software (GE Healthcare). The predicted MW of the different alternative splicing isoforms of each protein were obtained from UniProtKB database (37) and their pIs were calculated with ExPASy's compute pI/MW tool based on their aminoacidic sequence (38, 39). Observed protein targets of AAbs are represented by circles and the predicted basal isoforms by crosses in order to allow a rapid visual estimation of how much AAb-targeted isoforms differ from their basal (non-modified) proteins. All maps were represented in RStudio.

Functional Enrichment Analysis

Cytoscape (40) app ClueGO (41) was used to perform functional enrichment analysis of AAb targets. Human Gene Ontology Biological Function database from 18.05.2017 was used. Two-tailed hypergeometric test followed by Benjamini-Hochberg multiple comparison correction was employed. Only terms significantly enriched after multiple comparison correction ($p < 0.05$ and false discovery rate (FDR) < 0.05) are shown in the results section.

Other Statistical Analysis

Comparison of IgG binding from healthy and SCI individuals to proteins isolated from intact and injured spinal cord (shown in **Figure 1N**) was performed by two-way ANOVA in R Studio, using stat package (33).

RESULTS

IgGs Against Proteins Found in Damaged Spinal Cord Are Present in the Sera From Healthy Individuals and Their Levels Increase in SCI Patients

Serum IgG levels in SCI patients are significantly higher at 1 month after injury compared to healthy subjects, while IgM levels fail to reach statistical significance (**Figures 1A,B**). In experimental models, at the acute phase, total IgG and IgM serum levels increase after low thoracic lesions (3) and decrease after cervical lesions (16). Contrasting with these observations, in our study neither neurological level of injury nor severity affects total IgG or IgM serum levels (**Figures 1C–F**). To explore whether the increased serum IgG after SCI could be related to higher levels of autoantibodies (AAbs), we tested the immunoreactivity of sera samples IgG against rat spinal cord tissue. Confocal microscope settings were tuned to assure that any signal detected was specific, not attributable to background or unspecific staining from secondary antibodies. For this purpose, sections from rat spinal cord at 14 days after lesion were incubated only with the secondary anti-human IgG Ab used to detect IgG in human sera. This control immunostaining does not display any signal neither around the lesion epicenter when photomultiplier gain is set up at 750 mV, nor in the lesion epicenter (where autofluorescence is higher) with even less restrictive conditions (gain at 850 mV; **Figures 1G–H**). On the contrary, at the lower photomultiplier sensitivity setting −750 mV– specific signal is observed whenever human serum is tested (**Figures 1I–L**). Indeed, a weak although specific IgG signal is detected with sera from healthy control subjects on intact rat spinal cord, depicting mainly motor neurons in the ventral horn (**Figure 1I**). However, when sera from control subjects are incubated with injured spinal cord tissue sections instead of intact tissue, an unexpected stronger IgG signal against spinal cord antigens is observed (**Figure 1K**). In this case, cellular structures are observed consistent with neuronal bodies in the gray matter and axons and glia in the white matter. In comparison, with the same confocal settings, sera from SCI patients show a robust IgG signal against intact rat spinal cord, depicting motor neurons in the ventral horn as well as axons and glial profiles in the white matter (**Figure 1J**), which among other mechanisms could be related to the higher IgG levels in SCI sera (**Figure 1A**). Also, IgG from SCI patients strongly bind to injured spinal cord tissue (**Figure 1L**). Altogether, our results suggest that both healthy and SCI individuals carry IgG AAbs against spinal cord antigens, although AAb from healthy individuals are clearly detectable only when their sera are tested against injured spinal cord. To confirm this, we analyzed by western blot the binding of serum IgGs to proteins isolated from spinal cords of intact and injured rats at 1 and 28 days after lesion (**Figure 1M**). Our results show that IgGs from healthy control subjects display the same pattern of bands against protein extracts from both intact and injured rat spinal cords, although immunoreactivity is stronger against proteins isolated from injured spinal cord (**Figure 1M**, left panel). Similarly, IgGs from SCI patient's sera recognize the same pattern of bands in protein extracts from both intact and injured spinal cord (**Figure 1M**, right panel). Two-way ANOVA of Western blots quantification confirms that IgG immunoreactivity from SCI patients is significantly higher than that of IgGs from control individuals ($p = 0.006$; **Figure 1N**). Also, Student's *t*-tests confirms that IgG immunoreactivity from control subjects is significantly higher toward proteins isolated from injured spinal cords at 1 or 28 days after injury than toward proteins isolated from intact spinal cords (**Figure 1N**).

Serum IgGs Target Antigens Expressed on Neurons, Astrocytes, and Oligodendrocytes

Although serum IgGs clearly depict ventral horn motoneurons and white matter axons (**Figure 1**), we confirmed this by immunohistochemistry: **Figure 2** shows that IgGs from SCI patients bind to cellular structures positive for Neurofilament (NF) both in intact and injured spinal cord white matter. Interestingly, while IgGs from SCI patients do not bind to intact myelin surrounding white matter axons [determined as structures positive for Myelin Basic Protein (MBP)], they react with disorganized myelin adjacent to dystrophic axons on injured spinal cords (**Figures 2C,D,G,H**). Also, IgGs bind to cellular profiles negative for NF and MBP: as shown in **Figure 3**, patient's IgGs bind to cells expressing Glial Fibrillar Acidic Protein (GFAP, a marker of astrocytes), and Adenomatous Polyposis Coli (APC, a marker of oligodendrocytes) both in tissue sections of intact (**Figures 3A–F**) and injured spinal cord (**Figures 3G–L**). IgGs from healthy subjects also bind to neurons (see ventral horn motoneurons depicted in **Figure 1K**), astrocytes and oligodendrocytes in the injured spinal cord (**Figures 3M–R**).

Altogether, our results show that increased AAbs after SCI target neurons, astrocytes and oligodendrocytes in the spinal cord.

Autoantibodies Increased at the Acute Phase Are Naturally Occurring Antibodies Preferentially Directed Against Non-native Isoforms of 16 Different Proteins

We detected and quantify the binding of serum AAbs to their targets by Two Dimensional-Western blot (2D-WB) of sera against a pool of proteins extracted from pathological and control human spinal cords. Afterwards, we identified AAb targets by mass spectrometry (workflow schematized in **Supplementary Figure 1**). Thus, by 2D-WB we observed, again, that AAbs are present in the sera of both control and SCI subjects (**Supplementary Figure 2**) and bind to 173 different targets that correspond to isoforms/post-translational modifications of 37 different proteins (**Supplementary Table 2**). An overall increase in both IgG and IgM AAb levels after SCI compared with age- and gender-matched control individuals is observed (volcano plot representations of SCI/control

ratios are heavily skewed toward the right; **Figures 4A,B**), although only 47 IgG and 15 IgM AAbs reached statistical significance (*t*-test followed by multiple comparison correction by Benjamini-Yekutieli method under a false discovery rate (FDR) minor than 0.05; orange spots in **Figures 4A,B**). The increased IgGs and IgMs react against several isoforms/post-translational modifications of a limited number of proteins. Specifically, 15 different proteins are targets of IgG (**Figure 4C**) while 8 are targets of IgMs (**Figure 4D**). Seven out of eight targets of IgMs are also targeted by IgGs, so a total of 16 different IgG and IgM AAbs specificities are detected. Among these, anti-GFAP, -MBP and -G3P AAbs have been previously described to increase after SCI, but the remaining AAbs are, to our knowledge, for the first time described to augment in this study. Identification of AAbs against GFAP, MBP, neurofilament light (NFL) and neurofilament intermediate (NFM) confirms our immunohistochemical observations; i.e., astrocytes, oligodendrocytes and neurons are targets of AAbs after SCI. On the other hand, we have also identified targets whose expression is not restricted to the central nervous system [for example albumin (ALBU), hemoglobin subunit alpha (HBA) or hemoglobin subunit beta (HBB)]. The antigenic targets recognized by AAbs for which there are not significant differences between healthy and SCI individuals are shown in **Supplementary Table 2** and include actin cytoplasmic 1 (ACTB), fructose-biphosphate aldolase C (ALDOC), annexin A2 (ANXA2), annexin A5 (ANXA5), carbonyl reductase 1 (CBR1), 60 kDa heat shock protein (CH60), dihydropyrimidinase-related protein 2 (DPYL2), stress-70 protein (GRP75), heat shock-related 70 kDa protein 2 (HSP72), malate dehydrogenase (MDHC), moesin (MOES), nicotinamide riboside kinase-2 (NRK2), peripherin (PERI), peroxiredoxin-1 (PRDX1), peroxiredoxin-6 (PRDX6), phosphoglycerate mutase 1 (PGAM1), pyruvate kinase (KPYM), tubulin alpha-1C chain (TBA1C), tubulin beta-4A chain (TBB4A), tubulin beta chain (TBB5), and vimentin (VIME).

Consistent with the description of natural autoantibodies as directed against modified proteins, most of the 47 IgGs and 15 IgMs increased after SCI bind to isoforms that differ in their isoelectric point (pI) and/or molecular weight (MW) from the basal predicted values (**Figures 4E–G**; **Supplementary Figure 3**), suggesting that most of the AAbs that we have detected in control and SCI subjects are directed against proteins with alterations or post-translational modifications. In this regard, some AAbs elevated after SCI recognize proteins with a lower MW than predicted, as occurs with the increased anti-NFM IgM AAb, that recognize an isoform around 60 kDa whose peptidic sequence confirms that is a degraded fragment of the canonical 102 kDa NFM (**Figure 4E**). Other AAbs recognize modifications of targets that affect their pI, as anti-MBP IgGs, that are directed against more acidic forms than the native isoforms (**Figure 4F**), and other AAbs recognize isoforms with alterations affecting both their MW and pI, as occurs with the elevated anti-GFAP IgGs (**Figure 4G**; MW and pI of all other antigenic targets shown in **Supplementary Figure 3**). Overall, our results suggest that the AAbs increased after SCI are directed against degraded protein fragments and/or post-translational modifications of proteins.

If as our results suggest, AAbs increased after SCI are natural antibodies (AAb naturally present in healthy subjects), their augmentation should be the result of a secondary humoral response and, consequently, their increase should be detected very early after SCI. To test this, we had the opportunity of assessing sera at 2 $\pm$ 0.4 days after injury (mean and standard error) obtained from a subgroup of 11 patients among the 52 individuals studied at 1 month. We compared in these patients by paired *t*-test the AAb levels at 2 days with those at 1 month after injury. As shown in **Figure 4H**, all 47 IgG augmented at 1 month after injury are already increased at 2 days (no statistically significant differences are found between 1 month and 2 days), and the same is observed for 12 out of 15 IgMs (80%), supporting that most AAb increased at the acute phase of SCI are natural antibodies.

Endogenous IgG AAbs Against Spinal Cord Antigens Are Also Detected Very Early After Experimental SCI in Rats

Since our results show that AAbs against injured spinal cord are naturally present in the sera of healthy subjects, theoretically they should extravasate into the spinal cord once the blood-spinal cord barrier breaks after SCI and should be detectable at early stages, before new IgGs may be synthesized. We experimentally confirmed this in rats, observing endogenous IgGs in the injured spinal cord after 1 day, depicting cellular profiles at the lesion epicenter (**Figure 5A**) and on the most rostral spinal cord level affected by the lesion (**Figure 5B**). Target cells are located both in the gray matter (as the cell profile pointed out in the ventral horn in **Figure 5C**) and in the white matter (**Figure 5D**). However, as expected, endogenous IgGs cannot be detected in the spinal cord of intact rats (**Figure 5E**) and no signal is observed when tissue sections are incubated with an antibody against rabbit-IgG (signal is species-specific; **Figure 5F**). Therefore, in agreement with the results described above on AAbs after human SCI, naturally occurring IgG AAbs against injured spinal cord are also detectable in rodents.

Binding of Autoantibodies to Spinal Cord Proteins Increases After Injury Independently of Lesion Level and Severity

Cervical or high thoracic lesions impairs primary humoral responses to a greater extent than low thoracic or lumbar lesions (8, 15). However, secondary immune responses are not affected by injury level (17). Our results show that binding of IgG and IgM AAb to their targets is not affected by lesion level: only 1 IgM AAb against hemoglobin subunit alpha (HBA) is increased in paraplegics compared with tetraplegic patients (*t*-test p-value < 0.5 and FDR < 0.05, **Figures 6A,B**) while not a single AAb is significantly affected by lesion level when classifying patients based on whether lesion is above or below the 5th thoracic segment (**Figures 6C,D**).

Also, in contrast with previous reports, the severity of lesion does not have an overall effect on IgG or IgM AAb binding to their targets (**Figures 6E,F**). To further assess the effect of severity on AAbs after SCI, we analyzed whether AAb levels

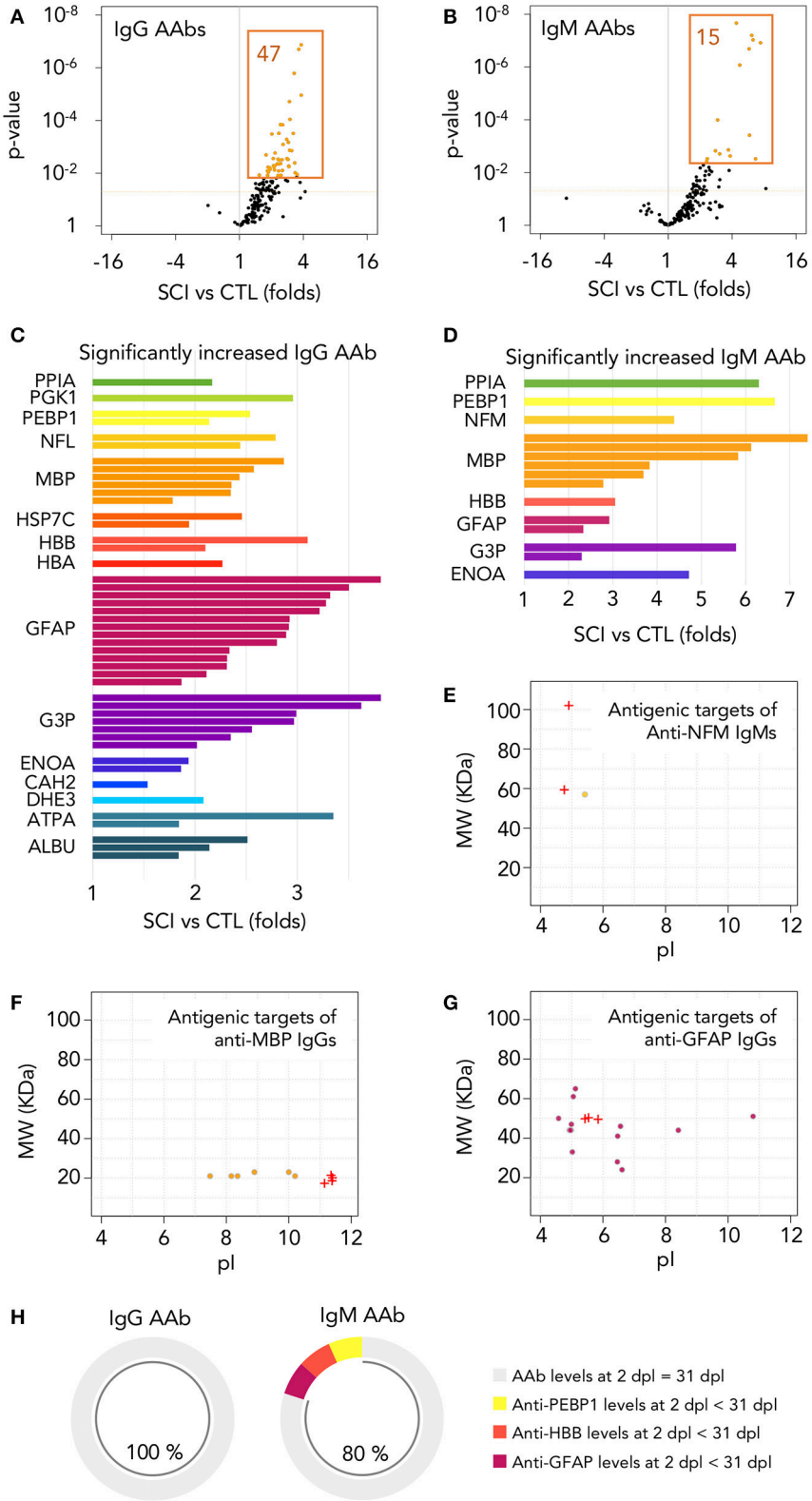

FIGURE 4 | Autoantibodies against modified central nervous system and peripheral proteins are rapidly increased after injury. **(A,B)** Among the 173 different IgG and IgM autoantibodies detected, 47 IgGs and 15 IgMs are significantly increased at 1 month after injury (*t*-test *p*-value < 0.05 and FDR < 0.05 after multiple comparison

(Continued)

FIGURE 4 | correction by Benjamini-Yekutieli method). Dotted horizontal orange line represents *t*-test *p*-value = 0.05. Orange-filled spots represent FDR < 0.05 after multiple comparison correction by Benjamini-Yekutieli method **(C,D)** Relative abundance and identity of the 47 IgGs and 15 IgMs increased in SCI patients. PPIA, peptidylprolyl isomerase 1; PGK1, phosphoglycerate kinase 1; PEBP1, phosphatidylethanolamine binding protein 1; NFL, neurofilament light; NFM, neurofilament intermediate; MBP, myelin basic protein; HSP7C, heat shock cognate 71 KDa protein; HBB, hemoglobin subunit beta; HBA, hemoglobin subunit alpha; GFAP, glial fibrillar acidic protein; G3P, glyceraldehyde-3-phosphate dehydrogenase; ENOA, alpha-enolase; CAH2, carbonic anhydrase 2; DHE3, glutamate dehydrogenase 1, mitochondrial; ATPA, ATP synthase subunit alpha, mitochondrial; ALBU, albumin. **(E–G)** Most of the autoantibodies increased after SCI are directed against proteins with modifications affecting their isoelectric point (pI) and/or their molecular weight (MW). Crosses represent the pI and MW of the basal isoforms produced by alternative splicing and circles represent the isoforms targeted by autoantibodies increased after injury. **(H)** Among the 47 IgGs increased at 1 month after injury, all of them reached similar levels at 2 days after injury (no statistically significant differences were found after paired *t*-test between AAb binding at both times), while the same is observed for 12 out of the 15 IgMs.

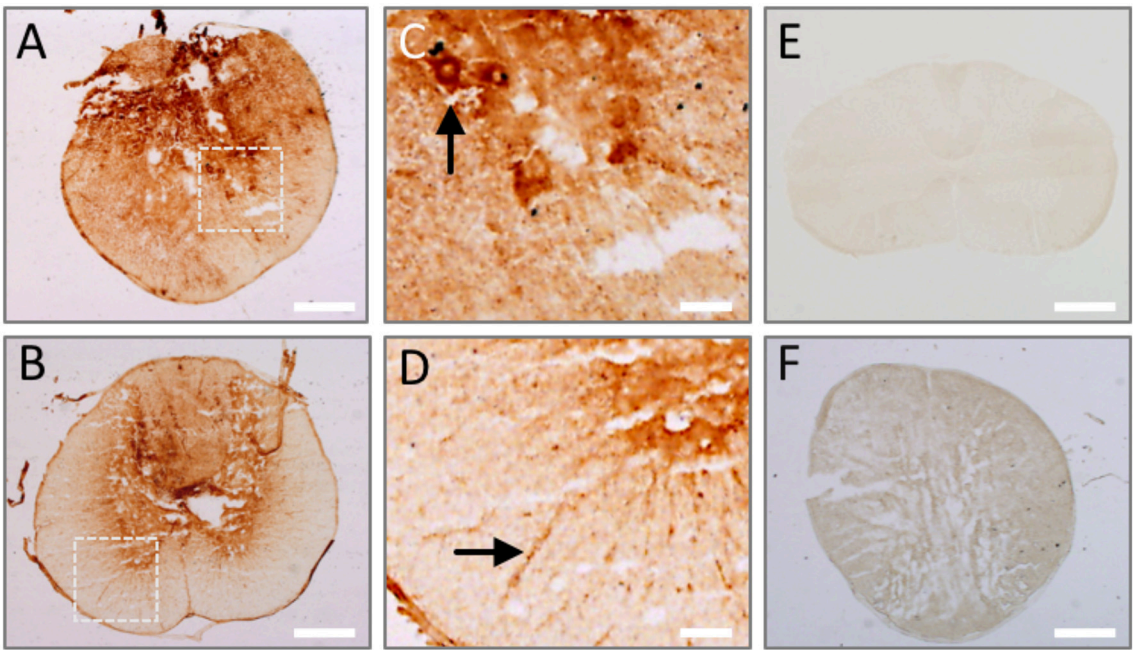

FIGURE 5 | Endogenous IgGs are detected in the spinal cord at 1 day after injury. Tissue sections from rat spinal cord at 1 day after injury were incubated with anti-rat IgG antibody and its binding was revealed by DAB-peroxidase reaction. Endogenous rat IgG is detected and depicts cellular profiles both in the lesion epicenter **(A)** and periphery **(B)**. **(C,D)** Show magnified details of inserts in **(A,B)**, where cell profiles in the ventral horn (arrow in **C**) and white matter (arrow in **D**) are clearly marked by endogenous IgG. **(E)** No endogenous IgG is observed in intact spinal cord. **(F)** IgG signal is species-specific, as shown by the absence of immune detection when using a secondary anti-rabbit IgG on injured spinal cord. Scale bar, 500 μm in **(A,B,E,F)**; 100 μm in C and D.

were different in a less severe SCI group, constituted by ten cervical AIS D central cord syndrome patients (CCS) (42). In fact, with the exception of slight anemia and thrombocythemia, the hematological values of CCS patients are like those of control subjects, while their counterparts cervical AIS A non-CCS SCI patients present more severe alterations that also affect to leukocyte populations (**Supplementary Table 3**). However, the levels of AAbs of CCS patients are like those of cervical AIS A non-CCS SCI patients (**Figures 6G,H**), reinforcing the observation that AAb levels are not affected by lesion severity.

Functional Enrichment Analysis of Autoantibody Targets

AAbs increased after SCI might be involved in pathophysiology (5) and/or might serve as biomarkers of underlying pathophysiological alterations (43, 44). To gain insight into the biological role of the increased AAbs, we analyzed the gene ontology biological function terms associated to their targets. This results in the statistically significant over-representation of functions that can be clustered in six major groups: (1) functions related with cytoskeleton organization (intermediate filaments, **Figure 7A**); (2) oxygen transport and removal of oxygen reactive species (**Figure 7B**); (3) energetic metabolism (**Figure 7C**); (4) albumin-related functions (**Figure 7D**); (5) response to virus (**Figure 7E**) and glutamate biosynthesis (**Figure 7F**).

DISCUSSION

Our results show that SCI induces a rapid increase in the levels of pre-existing natural IgG and IgM AAbs against modified isoforms from 16 different proteins independently of lesion level and severity. Among the antigenic targets, 13 are described for the first time and could become new therapeutic targets to test in future studies. Altogether, our results suggest that the origin

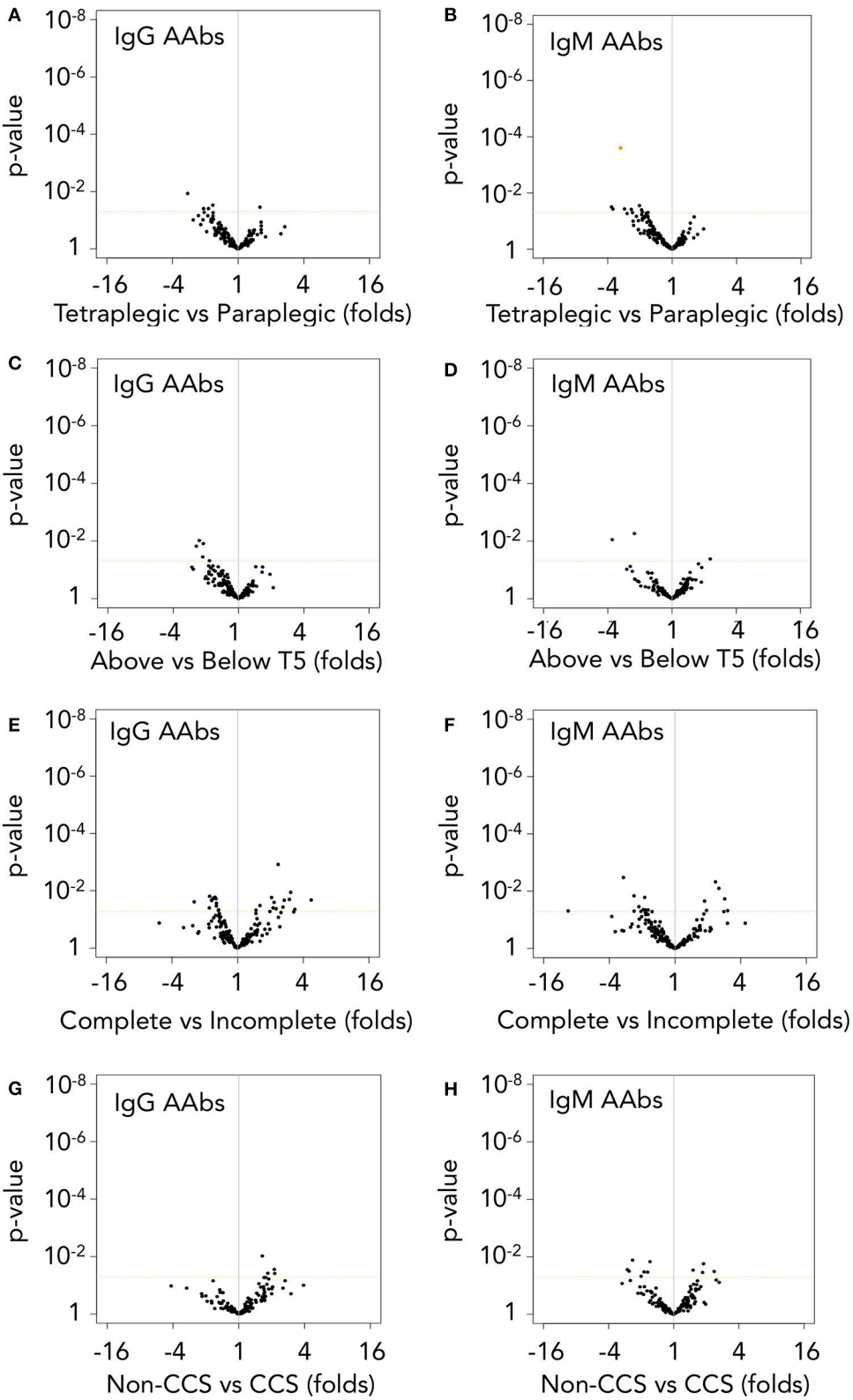

FIGURE 6 | Autoantibody levels are higher in spinal cord injury patients independently of lesion level and severity. **(A,B)** IgG and IgM AAb binding are not significantly different after performing *t*-test (*p*-value in *y*-axis) followed by false discovery rate (FDR) correction between tetraplegic and paraplegic patients, but for a single IgM

(Continued)

FIGURE 6 | AAb against HBA (orange-filled spot, **B**). **(C,D)** However, when classifying patients into those with lesions above or below T5 spinal segment, not a single AAb present different levels between the two groups. **(E,F)** Complete patients (AIS **A**) and incomplete patients (AIS **B–D**) do not show significant differences in IgG nor IgM AAb levels. **(G,H)** Comparison of cervical AIS D central cord syndrome patients (CCS, a less severe traumatic spinal cord injury) with their counterparts cervical AIS A patients (Non-CCS) also fails to found any statistically significant difference in AAb levels due to lesion severity. Dotted horizontal orange line represents *t*-test *p*-value = 0.05. Orange-filled spot represents FDR < 0.05 after multiple comparison correction by Benjamini-Yekutieli method.

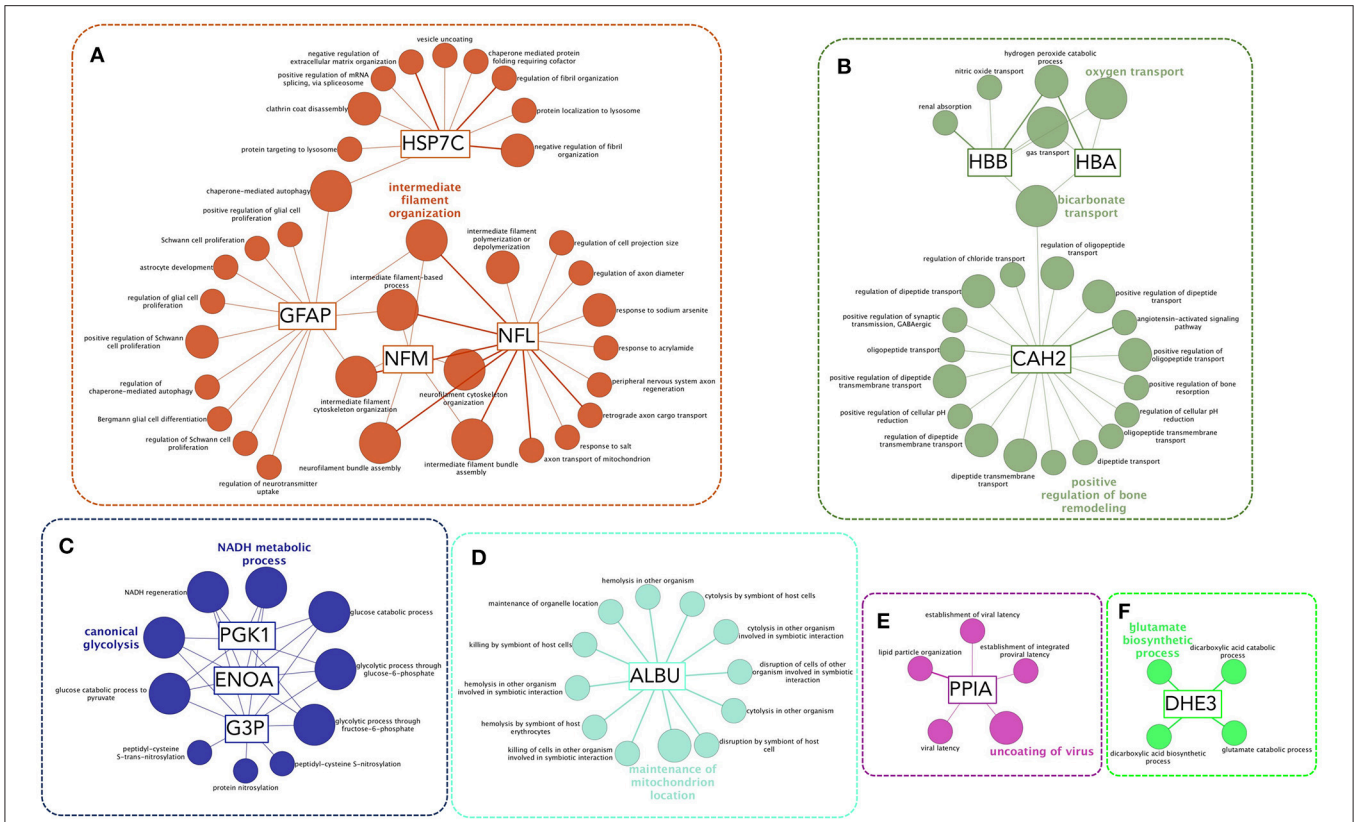

FIGURE 7 | Functional enrichment analysis of autoantibody targets reveals functions that could be affected by AAbs or that are affected by other injury-related processes and are being sensed by AAbs. Biological Function Gene Ontology terms associated to AAb targets are functionally organized into a network structure. Only significant terms after hypergeometric test followed by Benjamini-Hochberg multiple comparison correction are shown. Most significant terms of each functional group are highlighted. Six functional clusters may be established **(A–E)**: **(A)** a cluster formed by terms coupled to intermediate filaments-related functions, including proliferation and autophagy; **(B)** a cluster related to the control of oxygen radicals and oxygen transport, that is functionally related to a subcluster comprising CAH2 (carbonic anhydrase 2) functions, including bone remodeling; **(C)** a cluster including terms related to energetic metabolism, comprising glycolysis and NAD and ATP biosynthesis; **(D)** functions related to albumin; **(E)** functions related to PPIA (also known as cyclophilin A) that are involved in control of viral latency; **(F)** glutamate biosynthesis.

of antibody-mediated autoimmune responses after SCI is the expansion of previously formed natural AAbs.

Autoantibodies That Increase After SCI Exist Before Lesion

Natural AAbs are usually overlooked in healthy population mainly due to technical reasons (45). In this regard, several methodological details may explain why we observe a specific signal from AAbs in healthy individuals. For instance, our use of immunofluorescence and high sensitivity scanning to detect AAbs may uncover signals that remain below the threshold of detection by commonly techniques like chemoluminiscence or peroxidase reactions. But a major difference between our study and others may be the antigen collection employed to test for AAbs levels and specificity on 2D-WB experiments. Since we observed that sera from healthy individuals contain AAbs that are preferentially directed against antigens present in injured tissue (**Figure 1**), we made our antigen collection by pooling protein extracts from intact and pathological human spinal cord samples to include both normal proteins as well as potential neoepitopes arising after damage. Indeed, the recognition of protein neoepitopes induced by cell damage or stress is a major characteristic of natural AAbs (26, 27) and it has been shown that two neoepitopes arising after SCI are targeted by IgM AAbs naturally present in intact mice (46). Accordingly, our results show that most AAb increased after SCI are directed against isoforms with isoelectric points and molecular weights suggestive of degraded fragments or post-translational modifications. In this

regard, it should be considered that due to the lack of non-fixed human SCI tissue, the antigen collection is derived from control spinal cord samples as well as samples from patients with multiple sclerosis, Balo concentric lesions and lateral amyotrophic sclerosis. As explained before, these pathological samples were selected to include proteins that may be expressed "*de novo*" or modified by inflammation and neurodegeneration, events occurring also after traumatic SCI. We do not know whether exactly the same protein modifications are present in the spinal cord after traumatic injury, but the increase of AAbs against these modifications in SCI patients suggests that this might be the case.

Further supporting that AAbs increased after SCI are pre-existing AAbs is their rapid augmentation after injury, suggesting that they are part of secondary rather than primary humoral responses. In this regard, AAbs rise before new primary IgG responses may be detected (47), at the very acute phase in patients (**Figure 4**) and bound to their antigens in the spinal cord of rats at 1 day after lesion (**Figure 5**). Also supporting that this increase is due to secondary responses is the observation that it is independent of the neurological level of lesion, contrary to what occurs with primary humoral responses (17). Overall, our results do not distinguish whether the increased binding of IgG AAbs induced by SCI is just the result of increasing the levels of pre-existent natural AAbs or the result of maturating their affinity, but our data strongly support that increased AAbs at the subacute phase of SCI have their origin in naturally occurring AAbs.

The existence of naturally occurring IgG AAb in healthy subjects was reported in the early days of immunology and it has been a subject of research that challenges the classical view of the role of the immune system as merely defensive (44, 48, 49). Natural AAbs are originally present as germ line-encoded IgMs in newborns and as T-cell-dependent IgGs in adults (20, 22–24). Naturally occurring AAbs have been suggested to participate in physiological responses to tissue damage, such as cleaning of cellular debris, and their deficiency have been related to disease (26, 50, 51). Indeed, intravenous IgG (IVIg) preparations, which are used to treat both immunedepression and autoimmune disorders, are manufactured by pooling the serum IgGs from more than 1,000 healthy donors and are loaded with AAbs against a wide range of antigens (52). In this regard, a commercial human IVIg preparation containing IgG AAbs that bind to astrocytes, oligodendrocytes, microglia and neurons is therapeutic in experimental SCI (53), suggesting that endogenous naturally occurring AAbs might have a protective role immediately after SCI as well. A strong parallelism has been reported to occur in stroke, where pre-existing anti-NMDAR1 autoantibodies normally present in healthy subjects have an acute protective role if the blood brain barrier (BBB) is competent before occurrence of the ischemia (54, 55). However, when the BBB is chronically compromised before the insult, anti-NMDAR1 autoantibodies associates with larger lesion volumes. The access of pre-existing AAbs to the central nervous system after breakdown of the BBB has been also related with the pathogenesis of other neurological conditions (56). Unsolved issues worth of further research are whether the AAbs upregulated after SCI might also behave as a two-edged sword depending on the patient previous BBB estate and whether, additionally, these AAbs could convert into pathogenic due to their expansion, affinity maturation or other processes.

It should be also considered that although we have detected by immunohistochemistry, Western blot and 2D-Western blot the presence of these autoantibodies in the sera from sixteen intact subjects, their levels may vary depending on the age and gender of individuals (20). Further studies including a higher number of control subjects should be performed to assess the potential effect of these variables on the presence of naturally occurring autoantibodies throughout the population of healthy individuals.

Potential Implications of the Augmentation of Natural Autoantibodies After SCI

Regardless of whether the increased AAbs detected at this time are beneficial, pathogenic and/or could be used just as biomarkers of underlying processes, we performed a gene ontology based functional enrichment analysis to infer which cellular or physiological processes associated with the AAb targets could be more likely to be affected. This resulted in a significant enrichment of functions related to cytoskeleton organization, energetic metabolism, albumin-related functions, viral latency, transport of oxygen and bone remodeling as well as glutamate biosynthesis. Regarding the first set of these processes, cytoskeleton organization, it is well-known that SCI induces changes that affect glial and neuronal cytoskeleton, such as astrocytosis, axon dystrophy and myelin disorganization (clearly observable in **Figures 2**, **3**). Thus, the increase in anti-GFAP, anti-NFL and anti-NFM AAb levels could be contributing to cytoskeleton changes and/or reflecting that their targets are being modified. Of note, although anti-GFAP, anti-NFL and anti-NFM AAbs target intracellular proteins, it should not be discarded that AAbs could reach them *in vivo* (57, 58).

Patients also present an altered metabolic rate and several mechanisms have been proposed to explain it (59). The increase in anti-G3P, anti-ENOA, anti-KPYM, anti-PGK1, and anti-ATPA AAbs suggest that their target antigens are being modified after SCI –which could offer new perspectives on why metabolic alterations occur– and/or that metabolic alterations might be induced by an autoimmune response. In the same line of evidence, SCI patients develop osteoporosis and one of the gene ontology terms significantly enriched is "bone resorption," associated to CAH2.

Likewise, the levels of anti-PPIA AAbs increase. This protein, also known as cyclophilin A, is the target of the immunosupressor cyclosporine A (60), which opens the question of whether anti-PPIA AAbs might be endogenously mimicking cyclosporine. Functional enrichment analysis suggests that PPIA is, in addition, related to viral responses. In this regard, PPIA is necessary for the replication and reactivation of several virus, including human Cytomegalovirus (CMV) (61), proposed to be a general cause of immunosenescence (62, 63) and more specifically of immunosenescence after SCI (64). Whether anti-PPIA AAb levels could be an underlying mechanism of immunosenescence/immunodepression after SCI is worth of further studies.

It has been previously reported that SCI-induced immune deficiency syndrome (SCI-IDS) is rapidly established after lesion (65, 66) and is dependent on the lesion level and severity (10, 15). In an apparent contradiction, our results show that the opposite phenomenon, autoimmunity, is established at the same time and is independent of lesion level and severity. Thus, if both immunedepression and autoimmunity co-exist, they are likely to be independent phenomena. Supporting this, deficient humoral reactions are circumscribed to primary responses (responses against new antigens), while secondary antibody responses (already established responses) are preserved and thus not affected by the lesion level (17). Accordingly, as previously discussed, our results suggest that at least part of the AAb responses that are induced after SCI are secondary since (i) AAbs that augment after SCI are already present in healthy subjects at lower levels, (ii) all IgG AAbs that increase at 1 month after injury are also augmented before new IgG responses may occur, at 2 days after injury, and (iii) the level and severity of injury do not affect the levels of AAbs after SCI. Therefore, the AAb response at the subacute phase might be an independent phenomenon of immune depression because is the consequence of secondary immune responses of pre-existing AAbs.

CONCLUSIONS

Overall, our results show that a set of naturally occurring AAbs are expanded after SCI, which in the light of previous studies could help to explain why antibody-mediated autoimmunity initiates after SCI. Also, we report 16 antigenic targets that are involved in alterations that are known to occur after SCI, such as cytoskeleton remodeling, osteoporosis or metabolic rate change. Further studies are needed to elucidate the role of each AAb independently and the dynamics of their serum levels.

DATASET AVAILABILITY

The raw data supporting the conclusion of this manuscript will be made available upon request, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

AA-M conceived and designed the study. AA-M, LG, DG-O, OM, DM, and AE further refined the study. AA-M, LG, DG-O, and OM coordinated the study. LG, EV, MA, MAA, SC, RC, FT, RP, NS-B, DM, and AE are the treating physicians of the patients included in this study and together with OM collected samples and clinical data. CR coordinated the recruitment of healthy subjects and collected control samples. AA-M, DG-O, BP-T, GB-G, AA, and AT conducted and interpreted 2D-WB studies. GB and AA conducted and interpreted mass spectrometry studies. AA-M, DG-O, and BP conducted and interpreted immunohistochemistry studies. AA-M analyzed the data. AA-M, LG, DG-O, and EM-H interpreted the data. AA-M wrote the final manuscript with assistance from LG, DG-O, GB-G, and EM-H. All authors approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2018.02365/full#supplementary-material

REFERENCES

1. Mizrachi BY, Ohry A, Aviel A, Rozin R, Brooks ME, Schwartz M. Systemic humoral factors participating in the course of spinal cord injury. *Paraplegia* (1983) 21:287–93. doi: 10.1038/sc.1983.48
2. Hayes KC, Hull TCL, Delaney GA, Potter PJ, Sequeira KAJ, Campbell K, et al. Elevated serum titers of proinflammatory cytokines and CNS autoantibodies in patients with chronic spinal cord injury. *J Neurotrauma* (2002) 19:753–61. doi: 10.1089/08977150260139129
3. Ankeny DP, Lucin KM, Sanders VM, McGaughy VM, Popovich PG. Spinal cord injury triggers systemic autoimmunity: evidence for chronic B lymphocyte activation and lupus-like autoantibody synthesis. *J Neurochem.* (2006) 99:1073–87. doi: 10.1111/j.1471-4159.2006.04147.x
4. Davies AL, Hayes KC, Dekaban GA. Clinical correlates of elevated serum concentrations of cytokines and autoantibodies in patients with spinal cord injury. *Arch Phys Med Rehabil.* (2007) 88:1384–93. doi: 10.1016/j.apmr.2007.08.004
5. Ankeny DP, Guan Z, Popovich PG. B cells produce pathogenic antibodies and impair recovery after spinal cord injury in mice. *J Clin Invest.* (2009) 119:2990. doi: 10.1172/JCI39780DS1
6. Schwab JM, Zhang Y, Kopp MA, Brommer B, Popovich PG. The paradox of chronic neuroinflammation, systemic immune suppression, autoimmunity

after traumatic chronic spinal cord injury. *Exp Neurol.* (2014) 258:121–9. doi: 10.1016/j.expneurol.2014.04.023
7. Thietje R, Pouw MH, Schulz AP, Kienast B, Hirschfeld S. Mortality in patients with traumatic spinal cord injury: descriptive analysis of 62 deceased subjects. *J Spinal Cord Med.* (2011) 34:482–7. doi: 10.1179/2045772311Y.0000000022
8. Held KS, Steward O, Blanc C, Lane TE. Impaired immune responses following spinal cord injury lead to reduced ability to control viral infection. *Exp Neurol.* (2010) 226:242–53. doi: 10.1016/j.expneurol.2010.08.036
9. Bracchi-Ricard V, Zha J, Smith A, Lopez-Rodriguez DM, Bethea JR, Andreansky S. Chronic spinal cord injury attenuates influenza virus-specific antiviral immunity. *J Neuroinflamm.* (2016) 13:1–13. doi: 10.1186/s12974-016-0574-y
10. Brommer B, Engel O, Kopp MA, Watzlawick R, Müller S, Prüss H, et al. Spinal cord injury-induced immune deficiency syndrome enhances infection susceptibility dependent on lesion level. *Brain* (2016) 139:692–707. doi: 10.1093/brain/awv375
11. Zajarías-Fainsod D, Carrillo-Ruiz J, Mestre H, Grijalva I, Madrazo I, Ibarra A. Autoreactivity against myelin basic protein in patients with chronic paraplegia. *Eur Spine J.* (2012) 21:964–70. doi: 10.1007/s00586-011-2060-7
12. Hergenroeder GW, Moore AN, Schmitt KM, Redell JB, Dash PK. Identification of autoantibodies to glial fibrillary acidic protein in spinal cord injury patients. *Neuroreport* (2016) 27:90–3. doi: 10.1097/WNR.0000000000000502
13. Hergenroeder GW, Redell JB, Choi HA, Schmitt LH, Donovan W, Francisco GE, et al. Increased levels of circulating GFAP and CRMP2 autoantibodies in the acute stage of spinal cord injury predict the subsequent development of neuropathic pain. *J Neurotrauma* (2018). doi: 10.1089/neu.2018.5675. [Epub ahead of print].
14. Palmers I, Ydens E, Put E, Depreitere B, Bongers-Janssen H, Pickkers P, et al. Antibody profiling identifies novel antigenic targets in spinal cord injury patients. *J Neuroinflamm.* (2016) 13:1–11. doi: 10.1186/s12974-016-0713-5
15. Lucin K, Sanders V, Jones T, Malarkey W, Popovich P. Impaired antibody synthesis after spinal cord injury is level dependent and is due to sympathetic nervous system dysregulation. *Exp Neurol.* (2007) 207:75–84. doi: 10.1016/j.expneurol.2007.05.019
16. Ulndreaj A, Tzekou A, Mothe AJ, Siddiqui AM, Dragas R, Tator CH, et al. Characterization of the antibody response after cervical spinal cord injury. *J Neurotrauma* (2017) 34:1209–26. doi: 10.1089/neu.2016.4498
17. Oropallo MA, Held KS, Goenka R, Ahmad SA, O'Neill PJ, Steward O, et al. Chronic spinal cord injury impairs primary antibody responses but spares existing humoral immunity in mice. *J Immunol.* (2012) 188:5257–66. doi: 10.4049/jimmunol.1101934
18. Ibarra A, Martinez S, Reyes J, Meza-Lucas A, Mandujano A, Grijalva I, et al. Search for an IgG response against neural antigens in experimental spinal cord injury. *Neuroscience* (2000) 96:3–5. doi: 10.1016/S0306-4522(99)00541-2
19. Kidd JG, Friedewald WF. A natural antibody that reacts *in vitro* with a sedimentable constituent of normal tissue cells : II. Specificity of the phenomenon: general discussion. *J Exp Med.* (1942) 76:557–78.
20. Nagele EP, Han M, Acharya NK, DeMarshall C, Kosciuk MC, Nagele RG. Natural IgG autoantibodies are abundant and ubiquitous in human sera, and their number is influenced by age, gender, and disease. *PLoS ONE* (2013) 8:e60726: doi: 10.1371/journal.pone.0060726
21. Dighiero G, Lymberi P, Guilbert B, Ternynck T, Avrameas S. Natural autoantibodies constitute a substantial part of normal circulating immunoglobulins. *Ann N Y Acad Sci.* (1986) 475:135–45.
22. Mouthon L, Haury M, Lacroix-Desmazes S, Barreau C, Coutinho A, Kazatchkine MD. Analysis of the normal human IgG antibody repertoire. Evidence that IgG autoantibodies of healthy adults recognize a limited and conserved set of protein antigens in homologous tissues. *J Immunol.* (1995) 154:5769–78.
23. Merbl Y, Zucker-Toledano M, Quintana FJ, Cohen IR. Newborn humans manifest autoantibodies to defined self molecules detected by antigen microarray informatics. *J Clin Invest.* (2007) 117:712–8. doi: 10.1172/JCI29943
24. Madi A, Hecht I, Bransburg-Zabary S, Merbl Y, Pick A, Zucker-Toledano M, et al. Organization of the autoantibody repertoire in healthy newborns and adults revealed by system level informatics of antigen microarray data. *Proc Natl Acad Sci USA.* (2009) 106:14484–9. doi: 10.1073/pnas.0901528106
25. Avrameas S, Ternynck T, Tsonis IA, Lymberi P. Naturally occurring B-cell autoreactivity: a critical overview. *J Autoimmun.* (2007) 29:213–8. doi: 10.1016/j.jaut.2007.07.010
26. Lutz HU. Homeostatic roles of naturally occurring antibodies: an overview. *J Autoimmun.* (2007) 29:287–94. doi: 10.1016/j.jaut.2007.07.007
27. Chou MY, Fogelstrand L, Hartvigsen K, Hansen LF, Woelkers D, Shaw PX, et al. Oxidation-specific epitopes are dominant targets of innate natural antibodies in mice and humans. *J Clin Invest.* (2009) 119:1335–49. doi: 10.1172/JCI36800
28. Zelenay S, Moraes Fontes MF, Fesel C, Demengeot J, Coutinho A. Physiopathology of natural auto-antibodies: the case for regulation. *J Autoimmun.* (2007) 29:229–35. doi: 10.1016/j.jaut.2007.07.011
29. Poletaev AB, Morozov SG, Gnedenko BB, Zlunikin VM, Korzhenevskey DA. Serum anti-S100b, anti-GFAP and anti-NGF autoantibodies of IgG class in healthy persons and patients with mental and neurological disorders. *Autoimmunity* (2000) 32:33–8. doi: 10.3109/08916930008995985
30. Quintana FJ, Weiner HL. Understanding natural and pathological autoimmunity. *J Neuroimmunol.* (2006) 174:1–2. doi: 10.1016/j.jneuroim.2006.02.003
31. Arevalo-Martin A, Garcia-Ovejero D, Sierra-Palomares Y, Paniagua-Torija B, Gonzalez-Gil I, Ortega-Gutierrez S, et al. Early endogenous activation of CB1 and CB2 receptors after spinal cord injury is a protective response involved in spontaneous recovery. *PLoS ONE* (2012) 7:e49057. doi: 10.1371/journal.pone.0049057
32. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open source platform for biological image analysis. *Nat Methods* (2012) 9:676–82. doi: 10.1038/nmeth.2019.Fiji
33. R Core Team. *R: A Language and Enviroment for Statistical Computing*. Vienna: R Found Stat Comput (2017). Available online at: https//wwwR-project.org/
34. Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. *Ann Stat.* (2001) 29:1165–88. doi: 10.1214/aos/1013699998
35. RStudio Team. *RStudio: Integrated Development for R*. Boston, MA: RStudio, Inc (2016). Available online at: http//www.rstudio.com/
36. Shevchenko A, Wilm M, Vorm O, Mann M. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Anal Chem.* (1996) 68:850–8.
37. The UniProt Consortium. UniProt: the universal protein knowledgebase. *Nucleic Acids Res.* (2017) 45:D158–69. doi: 10.1093/nar/gkw1099
38. Bjellqvist B, Hughes GJ, Pasquali C, Paquet N, Ravier F, Sanchez JC, et al. The focusing positions of polypeptides in immobilized pH gradients can be predicted from their amino acid sequences. *Electrophoresis* (1993) 14:1023–31.
39. Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins M, Appel R, et al. Protein identification and analysis tools on the ExPASy server. In: Walker JM, editor. *The Proteomics Protocols Handbook.* Totowa, NJ: Humana Press (2005). pp. 571–607.
40. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* (2003) 13:2498–504. doi: 10.1101/gr.1239303
41. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al. ClueGO: a cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* (2009) 25:1091–3. doi: 10.1093/bioinformatics/btp101
42. Aito S, D'Andrea M, Werhagen L, Farsetti L, Cappelli S, Bandini B, et al. Neurological and functional outcome in traumatic central cord syndrome. *Spinal Cord* (2007) 45:292–7. doi: 10.1038/sj.sc.3101944
43. Quintana FJ, Merbl Y, Sahar E, Domany E, Cohen IR. Antigen-chip technology for accessing global information about the state of the body. *Lupus* (2006) 15:428–30. doi: 10.1191/0961203306lu2328oa
44. Cohen IR. Biomarkers, self-antigens and the immunological homunculus. *J Autoimmun.* (2007) 29:246–9. doi: 10.1016/j.jaut.2007.07.016
45. Abu-Shakra M, Shoenfeld Y. Natural hidden autoantibodies. *Isr Med Assoc J.* (2007) 9:748–9. doi: 10.1385/CRIAI:18:1:51
46. Narang A, Qiao F, Atkinson C, Zhu H, Yang X, Kulik L, et al. Natural IgM antibodies that bind neoepitopes exposed as a result of spinal cord injury, drive secondary injury by activating complement. *J Neuroinflamm.* (2017) 14:1–12. doi: 10.1186/s12974-017-0894-6

47. Siegrist C-A. Vaccine immunology. In: *Vaccines*. Amsterdam: Elsevier (2013). p. 14–32.
48. Nataf S. Autoimmunity as a driving force of cognitive evolution. *Front Neurosci.* (2017) 11:582: doi: 10.3389/fnins.2017.00582
49. Cohen IR, Cooke A. Natural autoantibodies might prevent autoimmune disease. *Immunol Today* (1986) 7:363–4. doi: 10.1016/0167-5699(86)90026-5
50. Fiskesund R, Stegmayr B, Hallmans G, Vikstrom M, Weinehall L, de Faire U, et al. Low levels of antibodies against phosphorylcholine predict development of stroke in a population-based study from northern Sweden. *Stroke* (2010) 41:607–12. doi: 10.1161/STROKEAHA.109.558742
51. Schwartz-Albiez R, Monteiro RC, Rodriguez M, Binder CJ, Shoenfeld Y. Natural antibodies, intravenous immunoglobulin and their role in autoimmunity, cancer and inflammation. *Clin Exp Immunol.* (2009) 158:43–50. doi: 10.1111/j.1365-2249.2009.04026.x
52. Kaveri SV. Intravenous immunoglobulin: exploiting the potential of natural antibodies. *Autoimmun Rev.* (2012) 11:792–4. doi: 10.1016/j.autrev.2012.02.006
53. Brennan FH, Kurniawan ND, Vukovic J, Bartlett PF, Käsermann F, Arumugam TV, et al. IVIg attenuates complement and improves spinal cord injury outcomes in mice. *Ann Clin Transl Neurol.* (2016) 3:495–511. doi: 10.1002/acn3.318
54. Zerche M, Weissenborn K, Ott C, Dere E, Asif AR, Worthmann H, et al. Preexisting serum autoantibodies against the NMDAR subunit NR1 modulate evolution of lesion size in acute ischemic stroke. *Stroke* (2015) 46:1180–6. doi: 10.1161/STROKEAHA.114.008323
55. Dahm L, Ott C, Steiner J, Stepniak B, Teegen B, Saschenbrecker S, et al. Seroprevalence of autoantibodies against brain antigens in health and disease. *Ann Neurol.* (2014) 76:82–94. doi: 10.1002/ana.24189
56. Levin EC, Acharya NK, Han M, Zavareh SB, Sedeyn JC, Venkataraman V, et al. Brain-reactive autoantibodies are nearly ubiquitous in human sera and may be linked to pathology in the context of blood-brain barrier breakdown. *Brain Res.* (2010) 1345:221–32. doi: 10.1016/j.brainres.2010.05.038
57. Hong CW, Zeng Q. Tapping the treasure of intracellular oncotargets with immunotherapy. *FEBS Lett.* (2014) 588:350–5. doi: 10.1016/j.febslet.2013.10.025
58. Sali AD, Karakasiliotis I, Evangelidou M, Avrameas S, Lymberi P. Immunological evidence and regulatory potential for cell-penetrating antibodies in intravenous immunoglobulin. *Clin Transl Immunol.* (2015) 4:e42. doi: 10.1038/cti.2015.18
59. Monroe MB, Tataranni PA, Pratley R, Manore MM, Skinner JS, Ravussin E. Lower daily energy expenditure as measured by a respiratory chamber in subjects with spinal cord injury compared with control subjects. *Am J Clin Nutr.* (1998) 68:1223–7.
60. Handschumacher RE, Harding MW, Rice J, Drugge RJ, Speicher DW. Cyclophilin: a specific cytosolic binding protein for cyclosporin A. *Science* (1984) 226:544–7.
61. Keyes LR, Bego MG, Soland M, St. Jeor S. Cyclophilin A is required for efficient human cytomegalovirus DNA replication and reactivation. *J Gen Virol.* (2012) 93:722–32. doi: 10.1099/vir.0.037309-0
62. Hadrup SR, Strindhall J, Køllgaard T, Seremet T, Johansson B, Pawelec G, et al. Longitudinal studies of clonally expanded CD8 T cells reveal a repertoire shrinkage predicting mortality and an increased number of dysfunctional cytomegalovirus-specific T cells in the very elderly. *J Immunol.* (2006) 176:2645–53. doi: 10.4049/jimmunol.176.4.2645
63. Moro-García MA, Alonso-Arias R, López-Vázquez A, Suárez-García FM, Solano-Jaurrieta JJ, Baltar J, et al. Relationship between functional ability in older people, immune system status, and intensity of response to CMV. *Age (Omaha)* (2012) 34:479–95. doi: 10.1007/s11357-011-9240-6
64. Pavlicek D, Krebs J, Capossela S, Bertolo A, Engelhardt B, Pannek J, et al. Immunosenescence in persons with spinal cord injury in relation to urinary tract infections -a cross-sectional study. *Immun Ageing* (2017) 14:1–14. doi: 10.1186/s12979-017-0103-6
65. Riegger T, Conrad S, Liu K, Schluesener HJ, Adibzahdeh M, Schwab JM. Spinal cord injury-induced immune depression syndrome (SCI-IDS). *Eur J Neurosci.* (2007) 25:1743–7. doi: 10.1111/j.1460-9568.2007.05447.x
66. Riegger T, Conrad S, Schluesener HJ, Kaps HP, Badke A, Baron C, et al. Immune depression syndrome following human spinal cord injury (SCI): a pilot study. *Neuroscience* (2009) 158:1194–9. doi: 10.1016/j.neuroscience.2008.08.021

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Autoimmune Glial Fibrillary Acidic Protein Astrocytopathy: A Review of the Literature

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Autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy is an autoimmune disease of the nervous system first defined in 2016. GFAP autoantibody, especially IgG that binds to GFAPα, has been reported in the cerebrospinal fluid (CSF) and serum of patients with GFAP astrocytopathy. The positive predictive value of GFAP antibody in the CSF is higher than in the serum. Tissue-based assay (TBA) and cell-based assay (CBA) are both recommended methods for the detection of GFAP antibody. GFAP astrocytopathy is accompanied by neoplasms, but the relationship between virus infection and GFAP astrocytopathy is unclear. GFAP antibody itself does not induce pathological changes; it is only a biomarker for the process of immune inflammation. The pathology of GFAP astrocytopathy in humans is heterogeneous. GFAP astrocytopathy is commonly diagnosed in individuals over 40 years old and most patients have an acute or subacute onset. Clinical manifestations include fever, headache, encephalopathy, involuntary movement, myelitis, and abnormal vision. Lesions involve the subcortical white matter, basal ganglia, hypothalamus, brainstem, cerebellum, and spinal cord. The characteristic MRI feature is brain linear perivascular radial gadolinium enhancement in the white matter perpendicular to the ventricle. Currently, there are no uniform diagnostic criteria or consensus for GFAP astrocytopathy and coexisting neural autoantibodies detected in the same patient make the diagnosis difficult. A standard treatment regimen is yet to be developed. Most GFAP astrocytopathy patients respond well to steroid therapy although some patients are prone to relapse or even die.

Keywords: astrocyte, antibody, meningoencephalitis, glial fibrillary acidic protein, astrocytopathy

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BACKGROUND

The novel concept of astrocytopathy, including neuromyelitis optica spectrum disorders (NMOSD) and autoimmune glial fibrillary acidic protein (GFAP) astrocytopathy, was recently suggested (1, 2). Unlike NMOSD characterized by aquaporin (AQP) 4 antibody, GFAP astrocytopathy is a meningoencephalomyelitis or limited form of meningoencephalomyelitis associated with IgG binding to GFAP. This disease usually involves the cerebra, meninges, spinal cord and optic nerve, and manifests as fever, headache, encephalopathy, myelitis, and abnormal vision (2–13).

HISTORY

Since 1991, reports of the clinical manifestations, images, and features of cerebrospinal fluid (CSF) in corticosteroid-responsive meningoencephalomyelitis, also known as chronic or subacute corticosteroid-responsive non-vasculitic autoimmune inflammatory meningoencephalitis (NAIM), have been published (14). Patients suffer from NAIM manifested as chronic/subacute encephalopathy or progressive dementia, and they tend to have severe abnormal findings by electroencephalography but no obvious changes by magnetic resonance imaging (MRI). Pathological analysis has revealed periangitis, gliosis, and T and B cell infiltration, with intact blood vessels in the brain parenchyma. As an autoimmune disease, NAIM is very sensitive to corticosteroid treatment.

Reports of zoonotic autoimmune disease are increasing. For example, N-methyl-D-aspartic acid (NMDA) antibody encephalitis was reported in polar bears (15). In addition, GFAP antibody was confirmed as a biomarker for necrotizing meningoencephalitis of pug dogs (16, 17). Classification by pathology includes granulomatous meningoencephalomyelitis (GME), necrotizing meningoencephalitis (NME), and necrotizing leukoencephalitis (NLE).

In 2016, a group led by Lennon (2, 3) in the Mayo Clinic published two important reports of meningoencephalitis in humans and termed the disorder autoimmune GFAP astrocytopathy. Our group started similar studies in 2013 and reported the pathological features of several cases of GFAP astrocytopathy. A long follow-up study has also been carried out. Several studies of GFAP astrocytopathy have been published to date (2–13) (**Table 1**).

DETECTION METHOD

AQP4 expressed on the endfeet of astrocytes was identified as a biomarker of NMOSD. The methods to detect this antibody include tissue-based assay (TBA), cell-based assay (CBA), flow cytometry, immunoblotting, and immunoprecipitation assay. Because GFAP is a cytosolic intermediate filament protein of astrocytes, methods for the detection of its antibody are limited. Currently, we can test for GFAP antibody by IF, CBA, and western blot.

Our previous studies (4, 5) and unpublished data used frozen sections of the hippocampus, brainstem and cerebellum and demonstrated that a characteristic IF pattern of GFAP-IgG was observed when IgG binds to the pia and subpia mimicking AQP4-IgG (**Figure 1**). However, the IF pattern differs from AQP4-IgG as follows (**Figure 1**): (1) it is prominent in the cell body and end-process located in all layers (molecular layer, white matter, and granular layer) whereas AQP4-IgG mainly binds to locations around the microvessels and the Virchow-Robin space; (2) in the cerebellum, it was detected in the molecular layers with a Bergmann radial pattern (**Figure 1A2**), which differs from the AQP4-IgG pattern that only has a microvessel profile (**Figures 1B1,B2**). Furthermore, the AQP4-IgG pattern is located at the border between two molecular layers with a Virchow-Robin space profile (**Figure 1B2**); and (3) in contrast to AQP4-IgG, no pattern-specific staining was detected in the stomach or kidney tissues when using CSF (**Figure 1**).

Tissue sections from different animals affect the results. In our study, sections from rats are better than from monkeys. Methods based on histology are sensitive but they cannot distinguish different subtypes of GFAP antibody. A disadvantage of this method is that it detects other protein antibodies coexpressed with GFAP in astrocytes, resulting in false positive results. For example, transglutaminase-6 is expressed in astrocytes, and its antibody is related to progressive multiple sclerosis (19).

CBA (**Figure 1B5**) is recommended for testing for AQP4 antibody and is the main method to test GFAP antibody. Because GFAP protein has more than eight isomers, using CBA to test all these isomer antibodies is very difficult. Testing of GFAP-IgG in the Mayo Clinic reported IgG reactive with the GFAPα isoform in 102 patients (100%) and GFAP-reactive IgG was lower than for other isoforms (3). A series of 22 patients from a European tertiary referral hospital reported a frequency of 100% binding to GFAPα, 63.6% to GFAPα and GFAPδ, and none to the GFAPδ isoform alone (7). Therefore, detection of GFAPα may be performed by CBA. However, in 19 Chinese patients who underwent CSF testing, we found fourteen cases that were GFAPα-IgG positive, and five cases who were only GFAPε-IgG positive, indicating that this result should be confirmed by other laboratories in the future.

Regarding the specificity of GFAP antibodies in the serum, more data are required for evaluation. Early studies using Enzyme-linked Immunosorbent Assay (ELISA) showed high concentrations of GFAP antibody in the serum of patients with Alzheimer's disease and trauma (20, 21). Therefore, the consequence of GFAP antibody in the serum of GFAP astrocytopathy is hard to determine. From our study and other recent reports, GFAP antibody in the CSF had a high specificity and sensitivity (2, 3, 5). Because ELISA has a number of disadvantages, such as low specificity for the detection of antibodies in some neuroimmune diseases (22), the influence of the methodology on the results cannot be ignored.

Earlier literature showed that GFAP antibodies were present in the CSF of some patients with MS (23). It was speculated retrospectively that patients previously diagnosed as MS but who did not meet the latest diagnosis criteria might have GFAP astrocytopathy. According to published studies (**Table 1**), patients with meningoencephalitis, myelomeningoencephalitis, encephalitis, myelitis, optic neuritis or autonomic nervous dysfunction with unclear reason should be tested for GFAP-IgG. TBA and CBA are both recommended methods.

Abbreviations: GFAP, autoimmune glial fibrillary acidic protein; IF, indirect immunofluorescence; CBA, cell-based assay; NMOSD, neuromyelitis optica spectrum disorders; AQP, aquaporin; CSF, cerebrospinal fluid; NAIM, non-vasculitic autoimmune inflammatory meningoencephalitis; MRI, magnetic resonance imaging; NMDA, methyl-D-aspartic acid; GME, granulomatous meningoencephalomyelitis; NME, necrotizing meningoencephalitis; NLE, necrotizing leukoencephalitis; LESCLs, longitudinally extensive spinal cord lesions; CNS, central nervous system; IVIG, immunoglobulin

TABLE 1 | Literatures of human GFAP astocytopathy.

Author	Year	Country	*n*	Pathological examination	Main findings
Fang et al. (2)	2016	USA	16	No	The first paper to described human GFAP astrocytopathy. They describe GFAP-IgG found in serum or cerebrospinal fluid that is specific for a cytosolic intermediate filament protein of astrocytes.
Flanagan et al. (3)	2017	USA	102	Yes	Retrospectively analyzed 102 GFAP-IgG positive patients. Specificity of serum and CSF testing, the clinical and radiological phenotype evaluate, the significance of coexisting antibodies, and therapy responses were reported. CSF-GFAPαa-IgG is highly specific for an immunotherapy-responsive autoimmune CNS disorder.
Yang et al. (4)	2017	China	7	Yes	To assess the treatment response in seven GFAP-IgG-positive patients with long-term follow-up. Some patients with GFAP astrocytopathy experienced a poor response to treatment although they received steroids and immunosuppressive agents,
Long et al. (5)	2018	China	19	Yes	To describe the clinical, radiological and pathological features in 19 patients with CSF-GFAP-IgG in CSF. The features of the neuropathology and immunopathology of GFAP astrocytopathies were perivascular inflammation and loss of astrocytes and neurons.
Yang et al. (6)	2018	China	10	Yes	To study overlapping syndromes in autoimmune GFAP astrocytopathy. Overlapping antibodies are common in GFAP astrocytopathy.
Iorio et al. (7)	2018	Italy	22	Yes	To report the clinical and immunological characteristics of 22 new patients with GFAP-IgG. GFAP autoimmunity is not rare. The clinical spectrum encompasses meningoencephalitis, myelitis, movement disorders, epilepsy and cerebellar ataxia. coexisting neurological and systemic autoimmunity are relatively common. Immunotherapy is beneficial in most cases.
Zarkali et al. (8)	2018	UK	1	No	To report a young man presenting with subacute meningoencephalitis and subsequent myelitis, and discuss the typical presentation and management of this severe but treatable condition.
Shu et al. (9)	2018	China	1	Yes	To examined brain biopsy sections from a patient with autoimmune GFAP astrocytopathy using hematoxylin and eosin and Luxol fast blue staining, and immunostaining with antibodies.
Martin et al. (10)	2018	USA	1	Yes	To report a 13-years-old girl with acute-onset meningoencephalitis and incidental finding of ovarian teratoma was found to have coexisting anti-NMDA-R and GFAP antibodies present in her cerebrospinal fluid.
Li et al. (11)	2018	China	1	No	To report a case of autoimmune GFAP astrocytopathy after herpes simplex viral encephalitis.
Dubey et al. (13)	2018	USA	90	Yes	This study demonstrates CSF GFAPα-IgG is a specific autoimmune meningoencephalomyelitis biomarker, with favorable corticosteroid response. Lack of response should prompt evaluation for co-existing NMDA-R-IgG or malignancy.
Sechi et al. (18)	2018	USA	13	No	This study found that spinal cord lesions in GFAP-IgG myelitis were commonly longitudinally extensive (≥80%) and centrally located. Compare to AQP4-IgG lesions, they were more subtle lesions with poorly defined margins and less swelling. In GFAP-IgG myelitis, spinal cord central canal, punctate or leptomeningeal enhancement was typical.

EPIDEMIOLOGY AND DEMOGRAPHICS

GFAP astrocytopathy is a rare condition even if probably under-diagnoses yet. Nowadays, epidemiological data of this disease is limited. GFAP astrocytopathy has a slight female predominance and tends to present in patients over 40 years old. Recently, children with GFAP astrocytopathy were reported with pediatric clinical presentations similar to adults (13). In a Chinese population, 19 patients were positive for GFAP antibodies, comprising 13 females and six males (ratio 2.17). The median age at disease onset was 54 years (range 23–73 years). A study by the Mayo Clinic reported 54% (55/102) were female and the median age at neurologic symptom onset was 44 years (range, 8–103 years). Iorio et al. found that the median age of their patients was 52 years (range: 6–80 years) and 13 patients were female (59%).

ETIOLOGY AND PATHOGENESIS

Currently, there is limited information regarding GFAP astrocytopathy and its pathogenesis. Similar to autoimmune encephalitis, GFAP astrocytopathy is also accompanied by a neoplasm. In a study by Lennon (2, 3), neoplasia was diagnosed in 34% patients, 66% of which were found within 2 years after neurologic onset. Twenty-two patients had neoplasms at disease onset, of which 15 were teratoma of the ovary, three were adrenal carcinoma, two were glioma, one was squamous cell carcinoma, and one was multiple myeloma. Results from Iorio et al. (7) showed that three patients had a history of tumors, and in our recent study, we found one patient with thyroid carcinoma, two patients with suspected meningeoma, and three with other benign tumors. Immunohistochemical staining of an ovarian teratoma from a GFAP astrocytopathy patient showed the cytoplasm of the glial process in neuronal tissue and epithelial cells reacted strongly with GFAP-IgG (7, 13). This supports the concept that ectopic expression in nervous system tissues contributes to the triggering of immune responses, such as that in autoimmune encephalitis (24).

Regarding infection, Lennon (2, 3) reported that 29% patients with GFAP antibody developed flu before neurologic symptoms and one patient had HIV. In a study by Iorio et al. (7), six (27%) patients had a premonitory symptom of flu, including one case of dengue fever. Two researchers from China found

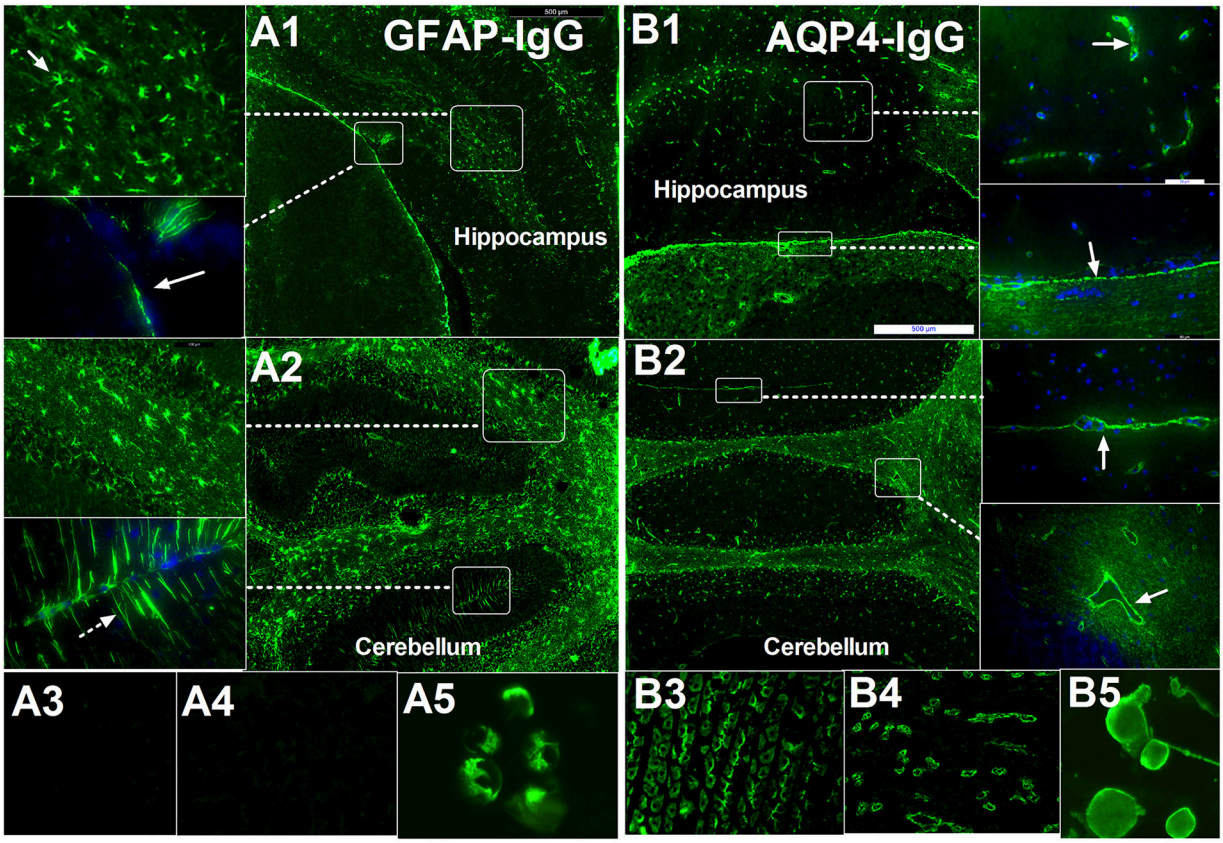

FIGURE 1 | Comparison of immunofluorescence-pattern between GFAP-IgG and AQP4-IgG. **(A1–A5)** IgG from patient with GFAP astrocytopathy. **(A1)** The IgG is bound to the foot process at pia (arrow) and astrocyte body in hippocampus. **(A2)** IF pattern in different layer of cerebellum: (1) The IgG is bound to the astrocyte body in different layer, especially white matter (arrow); (2) it was detected in molecular layers with bergmann radial pattern (arrow). **(A3,A4)** no pattern-specific staining was detected in the kidney and stomach tissue but positive for GFAP-transinfected HEK-293 cell. **(B1–B5)** IgG from a positive AQP4-IgG NMO patient. **(B1)** The IgG is bound to the cell with the foot process around the microvessels (arrow) in brain and pia (arrow). **(B2)** Anti-AQP4 pattern located at the border between two molecular layers with Virchow-Robin space profile (arrow) without bergmann radial pattern. **(B3–B5)** Pattern-specific staining was detected in the stomach, kidney tissue, and AQP4-transinfected HEK-293 cell.

evidence of herpes simplex infection in some patients (5, 11). The relationship between virus infection and GFAP astrocytopathy is unclear.

Currently, it is recognized that AQP4 antibody causes the loss of AQP4 antigen expression and the decrease of astrocyte numbers. AQP4 antibody binds to AQP4 expressed in the endfeet of astrocytes and activates complement. The downstream pathways involve excitatory amino acid transporter-2 endocytosis and result in demyelination and tissue necrosis. Using immunohistochemical analysis, we found the loss of AQP4 antigen expression and the decrease of astrocytes at different degrees in the lesions of patients with GFAP astrocytopathy. However, unlike AQP4, GFAP is an intracellular protein, thus it is difficult for GFAP antibody to interact with it. When a patient is positive for AQP4 antibody, the decrease in astrocyte numbers may be caused by AQP4 autoimmunity. However, this might also be secondary to other autoimmune diseases.

Animal model studies (25) indicated that GFAP astrocytopathy is mediated by GFAP peptide–specific cytotoxic T cells. The hypodermic injection of rat cerebral homogenate could induce vacuole or softening lesions in the cerebral cortex and $CD3^+$ T cells and microglial cells infiltrate the lesions. GFAP autoantibody can also be detected in the serum when $CD3^+$ T cells attach to GFAP-positive astrocytes. These pathological features of rats were consistent with those of canine NME. The above findings indicate that T cell-mediated immunity plays an important role in GFAP astrocytopathy.

NEUROPATHOLOGY

Histopathological research of human GFAP astrocytopathy is limited. No post-mortem report of this disease has been published. Histopathology analysis of the leptomeningeal biopsy specimen from one patient in a report by Iorio (6) revealed inflammatory changes in local tissues with mononuclear infiltration by macrophages and $CD8^+$ T cells. In a study by the Mayo Clinic, there was a rough description of the clinical characteristics including chronic inflammation and microglia activation without vasculitis. In China, five pathological studies reported data from stereotactic biopsy (4, 9). All the studies

showed inflammation around small vessels (**Figure 3**). The vascular wall was unaffected and no necrotic changes were found in tissues. T and B cells had infiltrated and scattered neutrophilic segmented granulocytes and eosinophils were present in the tissue. Many plasma cells were present in two cases. Histochemistry revealed two cases of severe AQP4 and GFAP dislocation, two cases of focal dislocation, and one case without dislocation. Therefore, the pathology of GFAP astrocytopathy in humans is heterogeneous. However, local stereotactic biopsy has disadvantages and does not reflect the whole picture of the disease.

In animal studies, a fatal necrotizing meningoencephalitis with GFAP-IgG as a biomarker was described (25). GME, NME, and NLE are idiopathic inflammatory diseases of the canine central nervous system (CNS) with differences in their clinical and pathological features (13). Histopathological necrosis was observed in both NME and NLE. Regarding the clinical features, GME involved a wide range of tissues including the cerebral cortex, cerebellum, brainstem, optic nerve, and spinal cord, and there was a marked elevation of white blood cells and protein levels in the CSF. Imaging revealed extensive abnormalities in the subcortical white matter. Histopathologically, GME was characterized by the accumulation of $CD3^+$ T cells and CD20 B cells around the vessels with an infiltration of plasma cells and monocytes. Neutrophils and granulomas were occasionally observed. Therefore, GME might be an equivalent to autoimmune GFAP astrocytopathy in humans.

CLINICAL FEATURES

Most patients have an acute or subacute onset, and progressively aggravated disease or have a relapse-remission pattern. Clinical manifestations include fever, headache, encephalopathy, involuntary movement, myelitis, abnormal vision, ataxia, mental disorder, epilepsy, abnormal autonomic nervous function, and other symptoms and signs of meningoencephalomyelitis. Reports of this disease were presented by the Mayo Clinic (USA), the Second Affiliated Hospital of Guangzhou Medical University (China), and Catholic University (Italy) (2–7, 13).

The report by Lennon (3) included 102 patients, whose most prominent clinical manifestations were encephalitis and meningitis (54.5%), followed by myelitis (10.5%) encephalomyelitis (8%), optic neuropathy, meningitis, ataxia, and meningoencephalomyelitis. Rare symptoms included epilepsy, dementia, and autonomic nervous dysfunction. In the study by Iorio, 10/22 (45%) cases manifested as encephalitis and meningitis. Other symptoms included ataxia, chorea, myelitis, optic neuritis, epilepsy, and dyskinesia (7). However, in a Chinese study, more patients suffered from myelitis (68.4%) and optic neuritis (63.2%), and longitudinally extensive transverse myelitis was more common (5). A recent study showed 10 children with positive GFAP-IgG had similar manifestations to previous studies in adults. Especially, seven cases with positive CSF-IgG all had a meningoencephalitis phenotype (13).

BIOLOGICAL AND IMAGING FEATURES

CSF in patients with GFAP astrocytopathy have high numbers of white blood cells ($>50 \times 106$/L), including lymphocytes, monocytes, and multinucleate cells. In addition, protein levels were increased to >1 g/L. The positive predictive value of GFAP antibody in the CSF is higher than in the serum. Patient serum and CSF in three cohorts (2, 3, 5, 7) contained other autoantibodies, such as NMDAR antibody, AQP4 antibody and other antibodies related to autoimmune diseases.

Brain MRI abnormalities are common (**Figure 2**). Lesions involved the subcortical white matter, basal ganglia, hypothalamus, brainstem, cerebellum, meninges, ventricle, and skull. In 32 Mayo Clinic patients, 18 of 32 (56%) had T2 hyperintensities and 21 of 32 (66%) had gadolinium enhancement (3). The characteristic pattern was brain linear perivascular radial gadolinium enhancement in the white matter perpendicular to the ventricle (**Figures 2A–D**), which was observed in 17 patients (53%). They also found a radial enhancement pattern in two patients with cerebellum abnormalities. Other enhancement patterns were observed in leptomeningeal ($n = 7$, 22%), sinuous demyelination ($n = 6$, 19%), and ependymal ($n = 3$, 9%) regions. Iorio et al. found hyperintense lesions on T2-weighted images consistent with inflammation present in 10 of 22 patients (45%), of which nine (41%) showed gadolinium enhancement. However, no cases with a characteristic pattern with radial enhancement were described in their study (7). In Chinese patients (5), 17 of 19 showed brain abnormalities (89.5%). Radial enhancing patterns were found in eight (42.1%,) and cortical abnormalities were found in four patients (21.1%). Positron emission computed tomography results from one patient showed extensive hypermetabolism in the cortex (5) and another patient showed hypometabolism in the basal ganglia (9). Other abnormalities occurred in the hypothalamus (15.8%), midbrain (36.8%), pons (68.4%), medulla (36.8%), cerebellum (36.8%), meninges (21.1%), skull (5.3%), and hydrocephalus (5.3%). The brain enhancement disappeared soon after treatment (4). Pathology showing meningitis and inflammation around small blood vessels indicated that the enhancement was caused by gadolinium leaking from the damaged blood-brain barrier (5). Following treatment, the blood-brain barrier was repaired rapidly and the enhancement disappeared.

Myelitis is commonly seen in GFAP astrocytopathy (2–8, 13). Among 71 patients with meningoencephalomyelitis from the Mayo Clinic, meningoencephalomyelitis phenotypes with myelitis were noted in 29 cases whereas myelitis alone was reported in two cases (combined: 43.6%, 31/71) (13). In early reports, of eight patients with MRI spine images available, six had longitudinally extensive myelitic abnormalities, one had a short myelitic lesion, and one had normal imaging. Linear-appearing central canal enhancement was noted in 21% of spinal cord magnetic resonance images (2). Abnormalities of the spinal cord were detected in four patients (4/22, 18.2%). In the study by Iorio (7), and three had a lesion that extended longitudinally for more than three contiguous vertebral segments. Recently, a study led by Sechi et al. (18) found that spinal cord lesions in GFAP-IgG

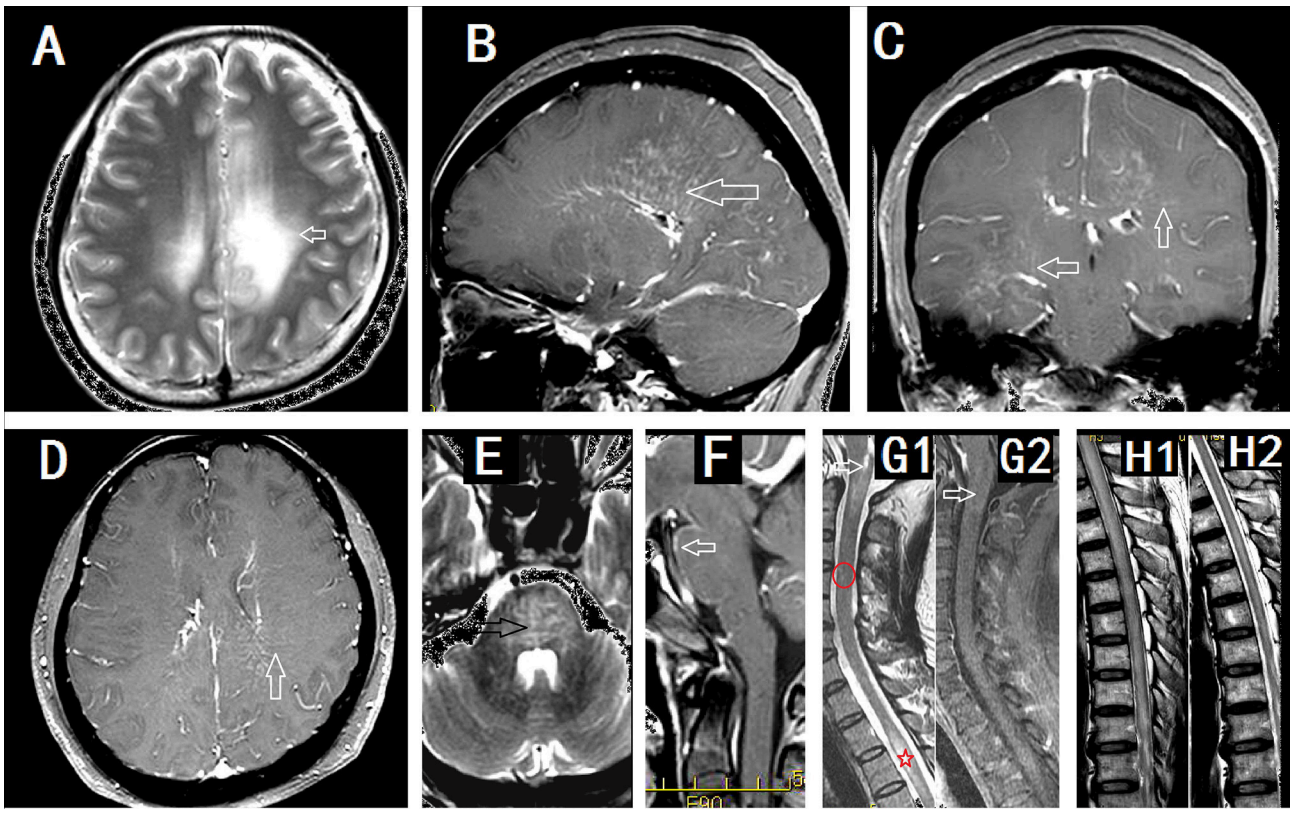

FIGURE 2 | Imaging findings in patients with GFAP astrocytopathy. **(A–D)** were from a female meningoencephalitis patient. **(A)** MR images showing extensive abnormalities in the white matter around the ventricle (arrow). **(B)** Sagittal section showed linear perivascular radial gadolinium enhancement in the white matter perpendicular to the ventricle(arrow). **(C,D)** Coronal section **(C)** and cross section **(D)** showed vessel-like enhancement (arrows). **(E)** and **(F)** from a male meningoencephalitis patient showed pons abnormality (black arrow) and pia enhancement (white arrow). **(G1)** and **(G2)** were from a female with myelitis. **(G1)** Cervical lesion extended to the area postrema of medulla(arrow), sparse cervical abnormality(red round area) and thoracic LESCLs (star marker). **(G2)** Slightly enhancement in medulla (arrow). **(H1)** and **(H2)** were from a male meningoencephalomyelitis patient. Longitudinal extensive lesions in the whole spinal cord **(H1)** and soon recovery after the treatment **(H2)**.

myelitis were commonly longitudinally extensive (≥80%) and centrally located. Compare to AQP4-IgG lesions, they were more subtle lesions with poorly defined margins and less swelling. In GFAP-IgG myelitis, spinal cord central canal, punctate or leptomeningeal enhancement was typical. Our study revealed more common spinal cord lesions. In Chinese cases, cervical and thoracic spinal cord MRIs were performed for 16 patients (84.2%, 16/19). Thirteen of these (81.25%) exhibited abnormal results, of which 11 patients had longitudinally extensive spinal cord lesions (LESCLs) (5). All cases showed central gray matter involvement in the spinal cord (**Figure 2G1**). LESCLs also were reported in some single cases (8).

DIAGNOSIS AND OVERLAPPING AUTOIMMUNE SYNDROMES

Currently, there are no uniform diagnostic criteria or consensus for GFAP astrocytopathy. The following questions require answers: (1) should the diagnosis of GFAP astrocytopathy be based on the presence of GFAP antibody in the CSF or on the criteria of meningoencephalomyelitis? We should not ignore that there have been some GFAP seropositivity in disease controls (e.g., astrocytoma) and perhaps it could occur occasionally as a secondary phenomenon. Should we term it collectively as "GFAP spectrum disorders?" It seems that the "classical phenotype" is meningoencephalitis or myelomeningoencephalitis, especially with positive CSF GFAP-IgG. However, the clinical scpectrum might be broader. (2) does the diagnosis of GFAP antibody negative astrocytopathy exist in patients manifesting as typical meningoencephalomyelitis, which was previously known as non-vascular autoimmune inflammatory meningoencephalitis?; (3) how should we classify other neurologic syndromes with GFAP antibody?; and (4) does GFAP astrocytopathy usually coexist with other autoantibodies that bind to astrocytes, neurons, and oligodendrocytes? For example, coexisting neural autoantibodies were detected in five patients in the study by Iorio et al. (7). Lennon found that 41 of 102 patients (40%) had coexisting antibodies in the CSF. N-methyl-D-aspartate receptor-IgG was the most common coexisting antibody. The other coexisting antibodies included aquaporin 4-IgG, anti-neuronal nuclear antibody-1, Purkinje-cell cytoplasmic IgG, leucine-rich

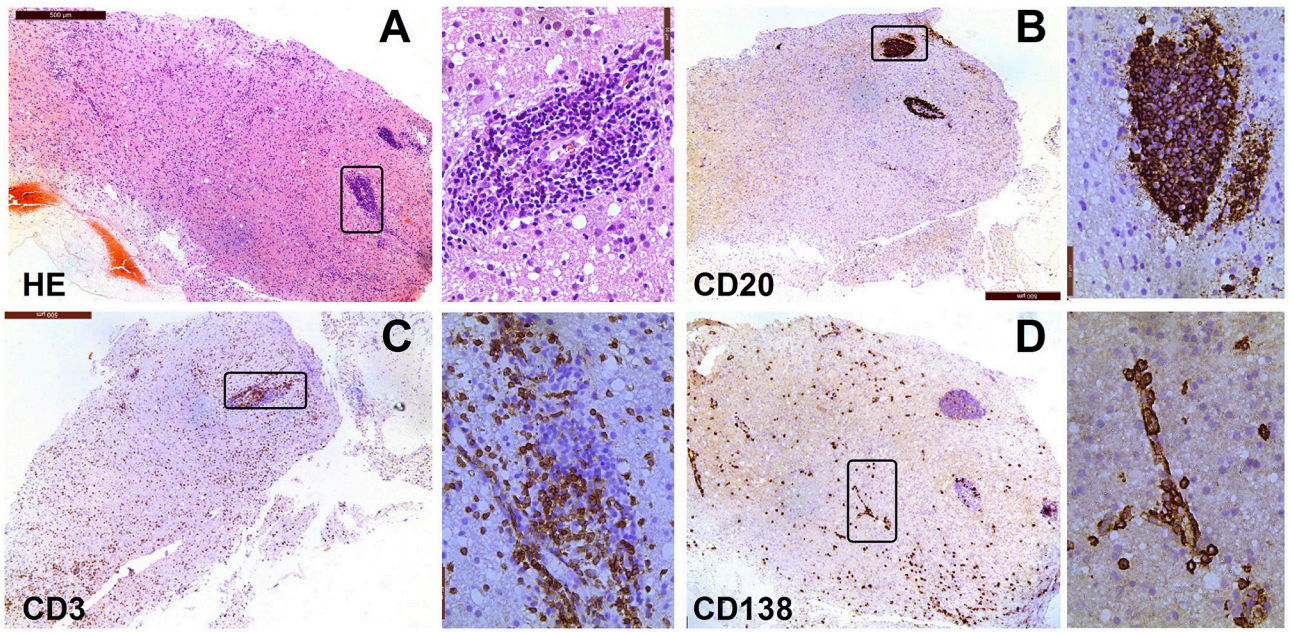

FIGURE 3 | Neuropathological features of a patient with encephalitis. The case involved a woman, whose age at onset was 69 years. In January 2015, she experienced psychosis, dyskinesia, persistent fever, and paralysis of a right-side limb. Antibody detection and biopsy were conducted before the IVMP and IVIG treatment. She then received treatment with methylprednisolone and azathioprine. She did not response well to this treatment and died of acute respiratory failure 1 year later. **(A)** Hematoxylin-eosin staining of brain biopsy tissue shows extensive infiltration of inflammatory cells; **(B)** Immunostaining for anti-CD20 is mainly around the vessels in lesions; **(C)** Immunostaining for anti-CD3 is sparse throughout the lesion, with some staining around the large vessel; **(D)** $CD138^{+}$ cells are present in perivascular, Virchow–Robin, and interstitial spaces.

glioma-inactivated protein 1-IgG, contactin-associated protein 2-IgG, and glutamic acid decarboxylase-65 isoform-IgG (3). In our study, 10 patients had GFAP antibody coexisting with other specific autoantibodies (5). Therefore, overlapping antibodies are common in GFAP astrocytopathy and make the diagnosis more difficult, especially at the initial attack. Care is needed when expanding the clinical spectrum based on serum positivity given it may be overlap with other autoimmune diseases.

TREATMENT AND PROGNOSIS

The treatment of GFAP astrocytopathy in the acute stage includes high-dose corticosteroids, intravenous immunoglobulin (IVIG), and plasma exchange. Long-term treatment includes oral steroids and immunosuppressants. About 70% of patients respond well to steroid therapy although some patients are prone to relapse. As a result, a standard treatment regimen has not been developed yet. According to our experience, some patients had a poor response to treatment or even died, and some patients were left with different degrees of functional disability (4).

Pathological biopsy showed that $CD138^{+}$ plasma cells were present in the brain lesions of the patients (5). This suggested that autoantibodies were synthesized in the brain, explaining why antibodies in the CSF are higher than in the peripheral blood. Furthermore, the continuous secretion of antibodies in the brain might affect the therapeutic effect. From our retrospective experience, pure steroid or IVIG therapy has a poor effect on patients with extensive brain or spinal cord lesions as well as high concentrations of GFAP antibodies in the CSF. Plasma exchange or immunosuppressive therapy may be more beneficial in the early stages of disease. This is also in accordance with the study in GME in dogs (26).

FUTURE PERSPECTIVES AND CONCLUSIONS

GFAP astrocytopathy is an autoimmune disease of the nervous system that requires further study regarding its etiology, pathology, mechanism, diagnosis, and treatment. In the report from the Mayo Clinic, most patients were Caucasian, while all subjects in our study were of Han nationality. Therefore, we should compare our patients with Caucasians regarding the prevalence, clinical manifestations, and prognosis.

To date, five of our patients have undergone pathological examination. However, stereotactic biopsy has some limitations. Invasive biopsy, or even autopsy, might provide more clear data regarding the immune mechanism involved in GFAP astrocytopathy. Currently, researchers consider GFAP antibody does not induce disease, but the main mechanism involved is still unknown. *In vitro* cell culture and animal experiments will enhance our understanding.

The clinical diagnosis of GFAP astrocytopathy needs to be resolved, especially in Overlapping Autoimmune Syndromes. Treatment in the acute stage includes glucocorticoids, IVIG, and plasma exchange. In addition to oral steroids the long-term use of immunosuppressive agents is appropriate for some patients.

AUTHOR CONTRIBUTIONS

FS and YL designed the concept, wrote the manuscript, and finalized it. WQ edited and read the final manuscript.

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REFERENCES

1. Lucchinetti CF, Guo Y, Popescu BF, Fujihara K, Itoyama Y, Misu T. The pathology of an autoimmune astrocytopathy: lessons learned from neuromyelitis optica. *Brain Pathol.* (2014) 24:83–97. doi: 10.1111/bpa.12099
2. Fang B, McKeon A, Hinson SR, Kryzer TJ, Pittock SJ, Aksamit AJ, et al. Autoimmune glial fibrillary acidic protein astrocytopathy: a novel meningoencephalomyelitis. *JAMA Neurol.* (2016) 73:1297–307. doi: 10.1001/jamaneurol.2016.2549
3. Flanagan EP, Hinson SR, Lennon VA, Fang B, Aksamit AJ, Morris PP, et al. Glial fibrillary acidic protein immunoglobulin G as biomarker of autoimmune astrocytopathy: analysis of 102 patients. *Ann Neurol.* (2017) 81:298–309. doi: 10.1002/ana.24881
4. Yang X, Liang J, Huang Q, Xu H, Gao C, Long Y, et al. Treatment of autoimmune glial fibrillary acidic protein astrocytopathy: follow-up in 7 cases. *Neuroimmunomodulation* (2017) 24:113–9. doi: 10.1159/000479948
5. Long Y, Liang J, Xu H, Huang Q, Yang J, Gao C, et al. Autoimmune glial fibrillary acidic protein astrocytopathy in Chinese patients: a retrospective study. *Eur J Neurol.* (2018) 25:477–83. doi: 10.1111/ene.13531
6. Yang X, Xu H, Ding M, Huang Q, Chen B, Yang H, et al. Overlapping autoimmune syndromes in patients with glial fibrillary acidic protein antibodies. *Front Neurol.* (2018) 9:251. doi: 10.3389/fneur.2018.00251
7. Iorio R, Damato V, Evoli A, Gessi M, Gaudino S, Di Lazzaro V, et al. Clinical and immunological characteristics of the spectrum of GFAP autoimmunity: a case series of 22 patients. *J Neurol Neurosurg Psychiatry* (2018) 89:138–46. doi: 10.1136/jnnp-2017-316583
8. Zarkali A, Cousins O, Athauda D, Moses S, Moran N, Harikrishnan S. Glial fibrillary acidic protein antibody-positive meningoencephalomyelitis. *Pract Neurol.* (2018) 18:315–9. doi: 10.1136/practneurol-2017-001863
9. Shu Y, Long Y, Chang Y, Li R, Sun X, Wang Y, et al. Brain immunohistopathology in a patient with autoimmune glial fibrillary acidic protein astrocytopathy. *Neuroimmunomodulation* (2018) 22:1–6. doi: 10.1159/000488879
10. Martin AL, Jolliffe E, Hertweck SP. Ovarian teratoma associated with coexisting anti-N-methyl-D-aspartate receptor and glial fibrillary acidic protein autoimmune meningoencephalitis in an adolescent girl: a case report. *J Pediatr Adolesc Gynecol.* (2018) 31:321–4. doi: 10.1016/j.jpag.2017.12.009
11. Li J, Xu Y, Ren H, Zhu Y, Peng B, Cui L. Autoimmune GFAP astrocytopathy after viral encephalitis: a case report. *Mult Scler Relat Disord.* (2018) 21:84–7. doi: 10.1016/j.msard.2018.02.020
12. Zekeridou A, McKeon A, Flanagan EP. A path to understanding autoimmune GFAP astrocytopathy. *Eur J Neurol.* (2018) 25:421–2. doi: 10.1111/ene.13527
13. Dubey D, Hinson SR, Jolliffe EA, Zekeridou A, Flanagan EP, Pittock SJ, et al. Autoimmune GFAP astrocytopathy: prospective evaluation of 90 patients in 1 year. *J Neuroimmunol.* (2018) 321:157–63. doi: 10.1016/j.jneuroim.2018.04.016
14. Caselli RJ, Boeve BF, Scheithauer BW, O'Duffy JD, Hunder GG. Nonvasculitic autoimmune inflammatory meningoencephalitis (NAIM): a reversible form of encephalopathy. *Neurology* (1999) 53:1579–81. doi: 10.1212/WNL.53.7.1579
15. Prüss H, Leubner J, Wenke NK, Czirják GÁ, Szentiks CA, Greenwood AD. Anti-NMDA receptor encephalitis in the Polar Bear (*Ursus maritimus*) Knut. *Sci Rep.* (2015) 5:12805. doi: 10.1038/srep12805
16. Talarico LR, Schatzberg SJ. Idiopathic granulomatous and necrotizing inflammatory disorders of the canine central nervous system: a review and future perspectives. *J Small Anim Pract.* (2010) 51:138–49. doi: 10.1111/j.1748-5827.2009.00823.x
17. Matsuki N, Takahashi M, Yaegashi M, Tamahara S, Ono K. Serial Examinations of anti-GFAP autoantibodies in cerebrospinal fluids in canine necrotizing meningoencephalitis. *J Vet Med Sci.* (2009) 71:99–100. doi: 10.1292/jvms.71.99
18. Sechi E, Morris PP, McKeon A, Pittock SJ, Hinson SR, Weinshenker BG, et al. Glial fibrillary acidic protein IgG related myelitis: characterisation and comparison with aquaporin-4-IgG myelitis. *J Neurol Neurosurg Psychiatry* (2018). doi: 10.1136/jnnp-2018-318004
19. Cristofanilli M, Gratch D, Pagano B, McDermott K, Huang J, Jian J, et al. Transglutaminase-6 is an autoantigen in progressive multiple sclerosis and is upregulated in reactive astrocytes. *Mult Scler.* (2017) 23:1707–15. doi: 10.1177/1352458516684022
20. Zhang Z, Zoltewicz JS, Mondello S, Newsom KJ, Yang Z, Yang B, et al. Human traumatic brain injury induces autoantibody response against glial fibrillary acidic protein and its breakdown products. *PLoS ONE* (2014) 9:e92698. doi: 10.1371/journal.pone.0092698
21. Tanaka J, Nakamura K, Takeda M, Tada K, Suzuki H, Morita H, et al. Enzyme-linked immunosorbent assay for human autoantibody to glial fibrillary acidic protein: higher titer of the antibody is detected in serum of patients with Alzheimer's disease. *Acta Neurol Scand.* (1989) 80:554–60. doi: 10.1111/j.1600-0404.1989.tb03926.x
22. Jarius S, Wildemann B. Aquaporin-4 antibodies (NMO-IgG) as a serological marker of neuromyelitis optica: a critical review of the literature. *Brain Pathol.* (2013) 23:661–83. doi: 10.1111/bpa.12084
23. Kaiser R, Obert M, Kaufmann R, Czygan M. IgG-antibodies to CNS proteins in patients with multiple sclerosis. *Eur J Med Res.* (1997) 2:169–72.
24. Seki M, Suzuki S, Iizuka T, Shimizu T, Nihei Y, Suzuki N, et al. Neurological response to early removal of ovarian teratoma in anti-NMDAR encephalitis. *J Neurol Neurosurg Psychiatry* (2008) 79:324–6. doi: 10.1136/jnnp.2007.136473
25. Park ES, Uchida K, Nakayama H. Establishment of a rat model for canine necrotizing meningoencephalitis (NME). *Vet Pathol.* (2014) 51:1151–64. doi: 10.1177/0300985813519115
26. Jung DI, Kang BT, Park C, Yoo JH, Gu SH, Jeon HW, et al. A comparison of combination therapy (cyclosporine plus prednisolone) with sole prednisolone therapy in 7 dogs with necrotizing meningoencephalitis. *J Vet Med Sci.* (2007) 12:1303–6. doi: 10.1292/jvms.69.1303

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Evidence for Innate and Adaptive Immune Responses in a Cohort of Intractable Pediatric Epilepsy Surgery Patients

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Brain-infiltrating lymphocytes (BILs) were isolated from resected brain tissue from 10 pediatric epilepsy patients who had undergone surgery for Hemimegalencephaly (HME) ($n = 1$), Tuberous sclerosis complex (TSC) ($n = 2$), Focal cortical dysplasia (FCD) ($n = 4$), and Rasmussen encephalitis (RE) ($n = 3$). Peripheral blood mononuclear cells (PBMCs) were also isolated from blood collected at the time of the surgery. Cells were immunostained with a panel of 20 antibody markers, and analyzed by mass cytometry. To identify and quantify the immune cell types in the samples, an unbiased clustering method was applied to the entire data set. More than 85 percent of the $CD45^+$ cells isolated from resected RE brain tissue comprised T cells; by contrast NK cells and myeloid cells constituted 80–95 percent of the $CD45^+$ cells isolated from the TSC and the FCD brain specimens. Three populations of myeloid cells made up >50 percent of all of the myeloid cells in all of the samples of which a population of HLA-DR^+ $CD11b^+$ $CD4^-$ cells comprised the vast majority of myeloid cells in the BIL fractions from the FCD and TSC cases. $CD45RA^+$ HLA-DR^- $CD11b^+$ $CD16^+$ NK cells constituted the major population of NK cells in the blood from all of the cases. This subset also comprised the majority of NK cells in BILs from the resected RE and HME brain tissue, whereas NK cells defined as $CD45RA^-$ HLA-DR^+ $CD11b^-$ $CD16^-$ cells comprised 86–96 percent of the NK cells isolated from the FCD and TSC brain tissue. Thirteen different subsets of CD4 and CD8 αβ T cells and γδ T cells accounted for over 80% of the $CD3^+$ T cells in all of the BIL and PBMC samples. At least 90 percent of the T cells in the RE BILs, 80 percent of the T cells in the HME BILs and 40–66 percent in the TSC and FCD BILs comprised

activated antigen-experienced (CD45RO$^+$ HLA-DR$^+$ CD69$^+$) T cells. We conclude that even in cases where there is no evidence for an infection or an immune disorder, activated peripheral immune cells may be present in epileptogenic areas of the brain, possibly in response to seizure-driven brain inflammation.

Keywords: brain, epilepsy, inflammation, peripheral immune cells, mass cytometry (CyTOF)

INTRODUCTION

It has been estimated that by 15 years of age, approximately one percent of children will have experienced at least one seizure (1). For reasons that may not always be understood ∼10% of children go on to develop medically refractory epilepsy, which is defined as a failure of two or more antiepileptic drugs to control seizures, and at least one seizure per month for ≥18 months (2). Recurrent seizures may severely impair a child's cognitive development leading to lifelong learning and behavioral difficulties. For children with drug-resistant epilepsy, surgery may be the only option to obtain seizure freedom, but will result in neurological deficits if the zone of resection involves eloquent cerebral cortex.

Many of the children who are candidates for epilepsy surgery suffer from rare neurological disorders including Rasmussen encephalitis (RE), Tuberous Sclerosis Complex (TSC), Focal Cortical Dysplasia (FCD), and Hemimegalencephaly (HME). RE patients present with partial (focal) seizures; magnetic resonance images (MRI) may indicate inflammation and atrophy in the affected cerebral hemisphere (3). The inflammation may spread through the affected cerebral hemisphere, but generally does not cross over to the contralateral hemisphere (4). Histopathological examination of resected brain tissue and brain biopsies show T cells in perivascular spaces, leptomeninges, and in small clusters scattered throughout the affected gray and white matter (5, 6). Clonally focused T cells have been found in resected RE brain tissue strongly implicating an antigen driven immune response in disease etiology (7–10).

In TSC patients, germ line and somatic dominant loss of function mutations in the genes encoding hamartin (TSC1) or tuberin (TSC2) can cause the development of benign tumors and, abnormally differentiated cortical neuronal progenitors that may cause focal seizures (11). Tuberin and harmatin are components of a complex that regulates the activity of the protein kinase, mTOR (12). Similarly, FCD and HME can be caused by activating somatic mutations in the MTOR gene, and in genes that regulate mTOR activity (13–15). Histopathological examination of resected brain tissue and analysis of RNA transcripts has shown that FCD and TSC lesions may be associated with an inflammatory reaction (11, 16–19).

In the present study we report on the characterization, by mass cytometry, of brain-infiltrating lymphocytes (BILs) isolated from surgical resections of epileptogenic tissue to treat FCD, TSC, and HME, as well as RE, and of peripheral blood mononuclear cells (PBMCs) prepared from blood collected at the time of surgery from the same cases. Immune cell profiling showed that activated T cells were present in brain tissue from all of the cases examined, and that the relative abundance of adaptive and innate lymphoid cells, and myeloid cells markedly differed between the RE and non-RE cases.

METHODS

Patient Cohort

Under UCLA IRB approval (IRB#18-000154) brain tissue and blood were collected at surgery as part of UCLA's Pediatric Epilepsy Surgery Program. All of the patients or their parents or legal guardians provided informed consent for the use of the surgical remnant and blood for research purposes. All specimens were collected using the same standard operating procedures. De-identified patient information including age at seizure onset, age at surgery, gender, and affected cerebral hemisphere was collected with informed consent (**Table S1**).

Isolation of Peripheral Blood Lymphocytes and Brain-Infiltrating Lymphocytes

PBMCs were isolated by density gradient centrifugation using Ficoll-Paque PLUS (GE Healthcare, Piscataway, NJ). BILs were isolated from collagenase-treated brain tissue by fractionation on a step gradient (20). Briefly, brain tissue was diced manually on ice in dissociation solution (HBSS with 20 mM HEPES pH 7.0, 5 mM glucose, and 50 U/ml penicillin/streptomycin). Tissue fragments were incubated with agitation at room temperature overnight in dissociation solution containing 0.5 mg/ml Type IV collagenase (Worthington Biochemical Corp., Lakewood, NJ) and 5% filtered human serum (Mediatech Inc., Manassas, VA). The dissociated tissue was fractionated on a 30%: 70% Percoll® (SigmaAldrich, St. Louis, MO) step gradient in RPMI containing 20 mM HEPES. PBMCs and BILs were cryopreserved in 90% human serum /10% DMSO.

Multiparameter Mass Cytometry

The panel comprised the following markers: CD45 (89Y or 154Sm), CD196 (141Pr), CD19 (142Nd), CD69 (144Nd), CD4 (145Nd), CD8 (146Nd), CD25 (149Sm), CD103 (151Eu), CD45RA (155Gd), CD183 (156Gd), CD56 (163Dy), CD45RO (164Dy), CD16 (165Ho), TCRγδ (168Er), CD3 (170Er), CD195 (171Yb), HLA-DR (174Yb), CD194 (175Yb), CD127 (176Yb), and CD11b (209Bi). All metal-tagged antibodies (Abs) were obtained from Fluidigm (San Francisco, CA) except the CD8 and TCRγδ antibodies, which were conjugated in-house. PBMCs and BILs were stained according to Fluidigm's protocol. In brief, cells were thawed, and washed in phosphate-buffered saline (PBS); prior to staining BILs were filtered through a 40 μm sieve to remove any aggregates. Cells were resuspended in 1 ml of PBS and stained for 5 min with 1 μM Cisplatin. After quenching the staining with Maxpar® cell staining buffer (Fluidigm), cells

were incubated with the cocktail of Abs in 100 μl of Maxpar® cell staining buffer for 30 min at room temperature. The Ab cocktail for staining PBMCs contained the CD45 Ab conjugated to Samarium 154 (154Sm) while the BIL Ab cocktail contained the CD45 Ab tagged with Yttrium 89 (89Y). Following two wash steps, cells were fixed overnight at 4°C in Maxpar® fixation and permeabilization buffer containing 0.125 μM Intercalator-Ir (Fluidigm). BILs and PBMCs from the same surgery were combined at this point and washed twice with Maxpar® cell staining buffer and a further two times with water. Having barcoded the BILs and PBMCs with a different metal-conjugated CD45 Ab it was possible to analyze them as single sample, thus the larger number of PBMCs served as a carrier for the smaller number of BILs that were isolated from brain tissue. Cells were resuspended in 10 percent EQ™ Four Element Calibration Beads (Fluidigm) containing Cerium (140/142Ce), Europium (151/153Eu), Holmium (165Ho), and Lutetium (175/176Lu). Samples were acquired on a Helios® cytometry time of flight (CyTOF) system (Fluidigm) at an event rate of 300–500 events/s. Post-acquisition data normalization was done using bead-based normalization in the CyTOF software. Prior to analysis, data were gated to eliminate normalization beads, debris, dead cells, and doublets.

The analysis of FCS files was initially carried out using Cytobank (21). For each surgery case, the marker expression on the BILs was resolved by first gating live singlets on CD45 (89Y). Conversely marker expression on PBMCs was resolved by first gating on CD45 (154Sm). The data were then split into two separate FCS files using software tools in Cytobank (21). To define subsets of immune cells in each BIL and PBMC population, the entire high dimensional dataset (comprising 20 FCS files) was converted into a matrix of pair-wise similarities by implementing the t-based stochastic neighbor embedding (t-SNE) algorithm, followed by a density-based clustering method (ClusterX) (22). The resulting 2-D plots from this procedure were exported to CorelDraw2017 as portable document format files (Corel Corporation, Ottawa, Canada). Individual FCS files were analyzed using FlowJo® software (FlowJo LLC, Ashland, OR); 2-D contour plots were exported as scalable vector graphic (svg) files to CorelDraw2017. The median level of expression of each marker was used to assign a phenotypic identity to each cluster. Heat maps were generated using Morpheus software (www.broadinstitute.org) and exported to CorelDraw2017 as svg files. Principal component analysis (PCA) was performed using PAST software (https://palaeo-electronica.org/2001_1/past/issue1_01.htm).

RESULTS

PBMCs and BILs isolated from 10 pediatric epilepsy cases were analyzed by CyTOF using a panel of antibodies designed to identify populations of adaptive lymphoid cells, innate lymphoid cells, and myeloid cells. Implementing the mass cytometry data analysis pipeline developed by Chen et al. (21) generated 46 clusters, corresponding to putatively distinct populations of $CD45^+$ cells (**Figure 1**). Clusters were classified as T cells, NK cells, and myeloid cells based on the relative expression of 19 immune cell markers. Immune cell clusters were divided into a $CD3^+$ ($n = 30$, median CD3 expression values of 4.648–6.283) and a $CD3^-$ group ($n = 16$, median expression values of 0.001–0.81). The $CD3^+$ group was subdivided into subsets of CD4, CD8, and γδ T cells based on the level of expression of these three phenotypic markers (**Figure 2**). The $CD3^-$ group was further divided into five NK cell subsets, ten myeloid and one B cell population based on the expression of CD56 and CD19 (**Figure 2**; **Table S2**).

Visual inspection of the t-SNE plots (**Figure 1**) showed that there were clear differences between the BILs from each surgical case compared with the corresponding PBMCs. On the other hand, the profiles of BILs from the two TSC (Case IDs 460 and 462) and the four FCD cases (Case IDs 475, 490, 494, and 495) appeared to be very similar and distinct from the three RE cases (Case IDs 472, 484, and 497), and dissimilar from the HME (Case ID 485), which appeared more similar to the RE cases. Principal components analysis based on the relative abundance of all of the clusters in each sample (percentages of $CD45^+$ cells) confirmed this observation, and also showed that the immune cell profiles of PBMCs from all of the cases were very similar (**Figure 3**). From the magnitude of the PCA loading values (**Table S3**), Clusters 1 and 8 accounted for the largest amount of variance in the first component, thus, the relative numbers of NK and myeloid cells in the BIL fractions appears to explain in large measure the observed difference between the TSC and FCD BILS compared with the RE and HME BILs. As summarized in **Figure 4**, $CD45^+$ cells from the FCD and TSC brain tissue specimens comprised far more NK cells and myeloid cells than $CD45^+$ RE BILs, which in agreement with previous studies (5, 6, 20, 23), were predominantly $CD8^+$ αβ T and γδ cells, The HME BILs comprised approximately equal numbers of T cells, NK cells and myeloid cells.

Three populations of myeloid cells (Clusters 1, 7, and 45) (**Figure 2**) made up >50 percent of all of the myeloid cells in all of the samples (**Figure 5**; **Table S4**). The Cluster 1 myeloid population comprised the vast majority of $CD11b^+$ cells in the BIL fractions from the FCD and TSC cases (**Figure 5**), whereas Cluster 7 $CD11b^+$ myeloid cells were more abundant in two of the three RE BIL fractions (Case IDs 472 and 484), and in all of the PBMCs (**Figure 5**). The only other marker that defined the Cluster 1 population was HLA-DR, thus it was not possible to assign a definitive phenotype to cells in this population, but they are likely to be macrophages. Likewise, Cluster 7 cells, which are $CD3^-$ $CD4^{lo}$ could constitute a dendritic cell or monocyte population, but additional markers are also required to adequately define this population.

Cluster 3 NK cells are $CD56^+$ $CD45RA^+$ HLA-DR^- $CD11b^+$ $CD16^+$ (**Figure 2**), and constituted the major population of NK cells in the blood from all of the cases (**Figure 5**). This subset also comprised the majority of NK cells that were found in BILs from the resected RE and HME brain tissue (**Figure 5**). By contrast, Cluster 8 NK cells, defined as $CD56^+$ $CD45RA^-$ HLA-DR^+ $CD11b^-$ $CD16^-$ (**Figure 2**) comprised 86–96 percent of the NK cells isolated from the FCD and TSC brain tissue (**Figure 5**; **Table S4**). We confirmed the existence of Cluster 3

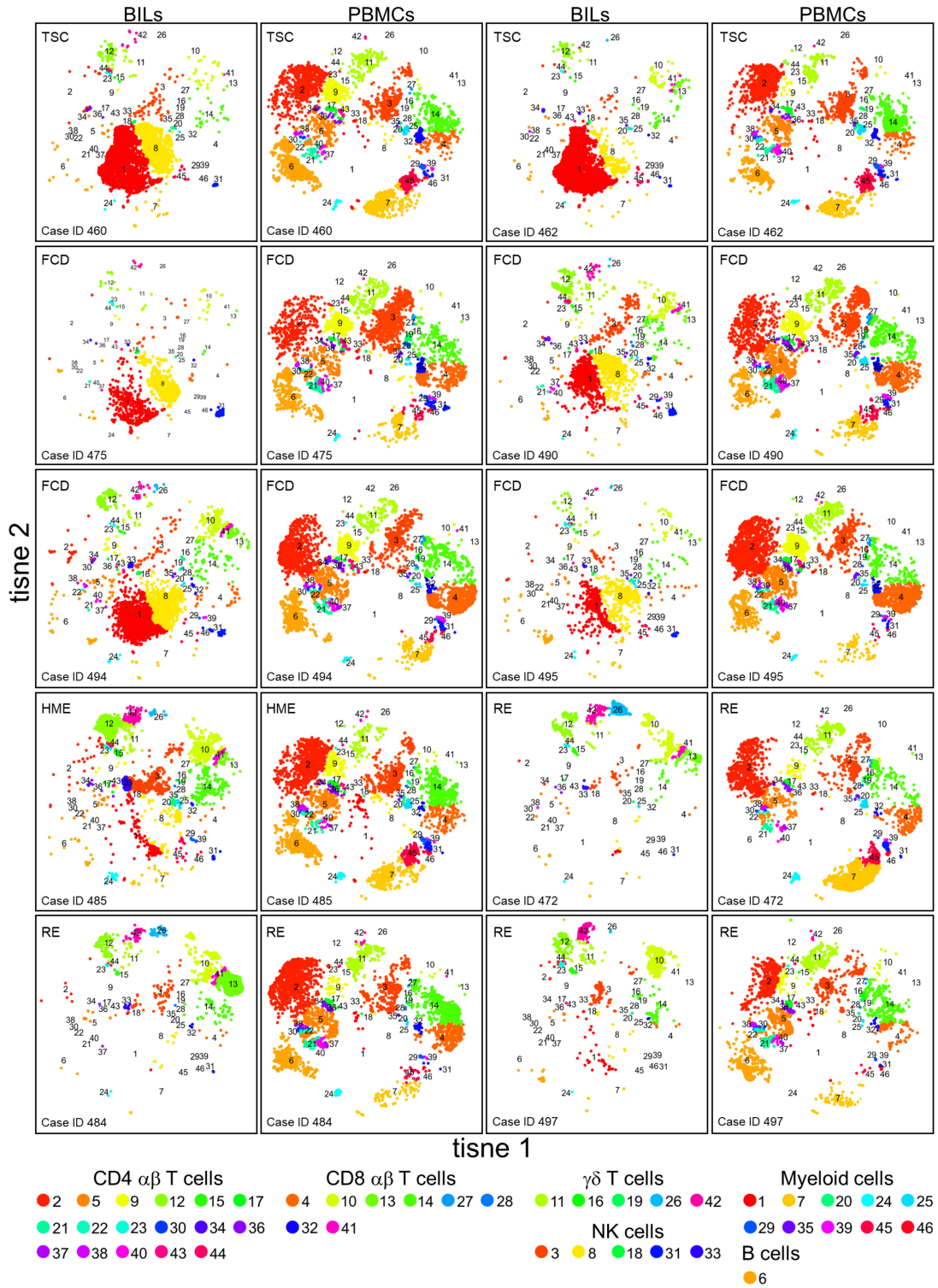

FIGURE 1 | tSNE plots showing the relative number of different immune cells in BILs and PBMCs from the pediatric epilepsy surgeries. The expression of 20 immune cell markers was analyzed by CyTOF. To define subsets of $CD45^{+}$ cells in each BIL and PBMC population, the entire high dimensional dataset (comprising 20 FCS files) was converted into a matrix of pair-wise similarities by implementing the t-based stochastic neighbor embedding (t-SNE) algorithm, followed by a density-based clustering method (ClusterX). The clusters were assigned as either T cells, NK cells, myeloid cells, or B cells based on the median expression values of specific immune cell markers (CD3, CD4, CD8, TCR γδ, CD11b, CD56, and CD19).

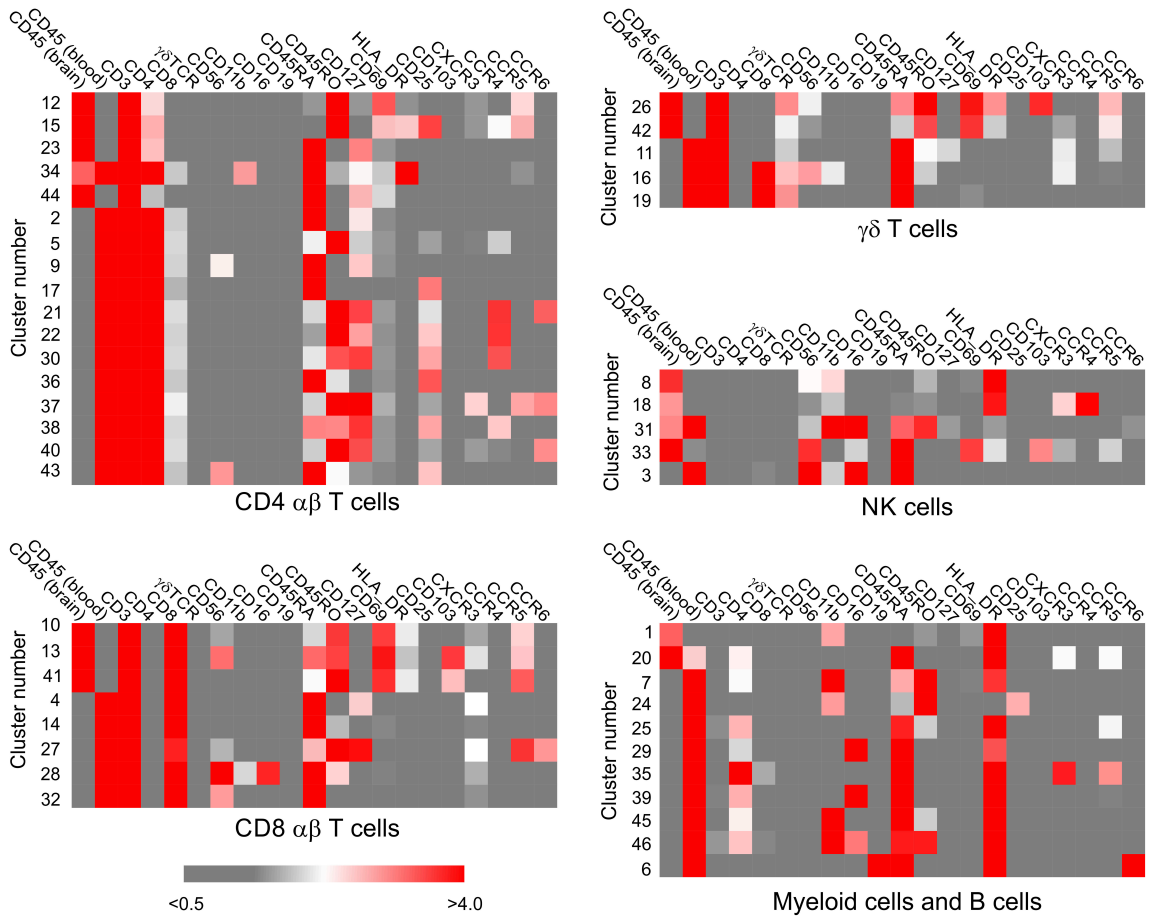

FIGURE 2 | Assignment of immune cell phenotypes. The median expression values of 19 immune cell markers, calculated by the Cytofit software, were used to assign a phenotype to each cluster of CD45$^+$ cells (**Table S2**). The data were first separated into CD3$^+$ and CD3$^-$ clusters, and the CD3$^+$ populations were further subdivided into CD4$^+$, CD8$^+$, γδ subsets. The CD3$^-$ populations were categorized as myeloid, natural killer cell, or B cell based on the expression of CD56 and CD19. Heat maps generated from the median expression values included all the markers that were expressed on cells in the CD3$^+$ CD4$^+$, CD3$^+$ CD8$^+$, CD3$^+$ γδ$^+$, CD3$^-$ CD56$^+$, CD3$^-$ CD19$^{+/-}$ clusters, respectively. The median expression value of the two different CD45 antibody metal conjugates used to stain the PBMC and BIL fractions reflects the relative number of PBMCs and BILs in each cluster.

and 8 NK cell populations by manually curating the original FCS files from FCD case 490 using the FlowJO software package (**Figure S1A**).

Six subpopulations of CD4$^+$ αβ T cells (Clusters 2, 5, 9, 12, 15, and 23), four of CD8$^+$ αβ T cells (Clusters 4, 10, 13, and 14), and three subsets of γδ T cells (Clusters 11, 26, and 42) accounted for over 80% of the CD3$^+$ T cells in all of the BIL and PBMC samples, although there were marked differences in the proportion of the different T cell subsets between the BIL and PBMC samples (**Figure 5**; **Table S4**). Clusters 2, 9 and 23 CD4$^+$ T cells constitute naïve T cell populations (CD45RA$^+$ CD45RO$^-$ CD127^{int}) (**Figure 2**); different median levels of expression of CD4 and CD127 appear to account for the generation of two clusters of naïve conventional T cells (Clusters 2 and 23), whereas expression of CD56 by Cluster 9 CD45RA$^+$CD45RO$^-$CD4$^+$ T cells indicates that they may be unconventional NKT cells.

Clusters 5 and 15 define two different subpopulations of antigen-experienced CCR4$^+$ CD4$^+$ T cells (**Figure 2**). Based on the expression of CD25, the interleukin-2 receptor α chain (IL-2R) and lack of CD127, the interleukin-7 receptor α chain (IL-7R), Cluster 15 cells appear to be conventional regulatory T cells (Tregs) (24), and were found almost exclusively in the BIL fractions (**Figure 5**). Additional minor populations of CD45RO$^+$ CD25$^+$ CCR4$^+$ CD4 T cells (Clusters 21, 22, 30, and 38) found predominantly in the blood may also be Tregs (**Figure 2**). Cluster 5 CD4 cells were found in both the brain and blood, and may be both IL-7 and IL-2 dependent (**Figures 2**, **5**). Cluster 12 defined an activated effector memory population of CD4 T cells (CD45RO$^+$ HLA-DR$^+$ CD69$^+$) (**Figure 2**) that was found in all of the BIL fractions (**Figure 5**). These cells also expressed the chemokine receptors CXCR3 and CCR5 thus may have trafficked to the epileptogenic brain area that was resected, possibly due to local inflammation (25).

Cluster 4 CD8 T cells were primarily found in the PBMC fractions and appear to correspond to an activated unprimed subtype (CD45RA$^+$ CD127$^+$) since they also expressed the

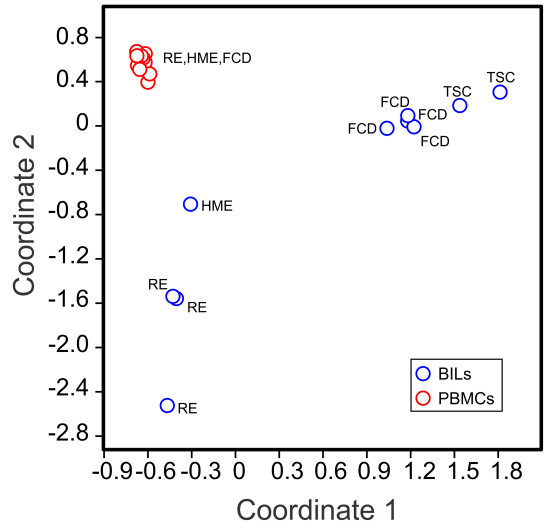

FIGURE 3 | Clustering of BILs and PBMCs from pediatric epilepsy surgeries. The relative number of cells in each cluster in each sample (as a percentage of the total number of $CD45^+$ cells analyzed in each sample) was used to implement a principal component analysis (PCA). The first two components could account for 65.72% of the total variance in the data set. The 2D PCA plot shows that the BIL fractions are clearly different from the corresponding PBMCs, and that BILs from the RE and HME cases are distinct from those from the FCD and TSC cases.

chemokine receptor CXCR3 (26). A $CD45RA^+$ $CD45RO^{lo}$ subpopulation (Cluster 14) made up most of the remaining CD8 T cells in the blood (**Figures 2**, **5**; **Table S4**). Cells in Clusters 10 and 13 appear to be activated ($CD69^+$ $HLA\text{-}DR^+$) effector memory subtypes, and were found almost exclusively in the brain (**Figures 2**, **5**). Based on the marker panel used in this study, the only difference between these two clusters was the expression of the resident memory marker CD103. Fewer Cluster 13 $CD8^+$ resident memory T cells (T_{RM}) were found in the brain compared with Cluster 10 T cells with one exception, Case ID 484, a RE patient. In agreement with previous work (27), the majority of the $CD8^+$ T cells isolated from resected brain tissue from this patient were T_{RM} cells. Likewise, the majority of γδ T cells isolated from a second RE case (Case ID 472) were T_{RM} cells (Cluster 26, **Figure 5**) (27). Cluster 26 and Cluster 42 γδ T cells expressed the activation markers CD69 and HLA-DR and were almost exclusively found the brain (**Figures 2**, **5**). These two activated subsets comprised 88–98 percent of the γδ T cells in the RE BIL fractions, whereas a third subset, Cluster 11, a $CCR5^+$ $CXCR3^+$ effector memory population ($CD45RA^+$ $CD45RO^{int}$ $CD127^+$) contributed up 70 percent of the γδ T cells in the other cases (**Table S4**). The Cluster 11 subset also made up the vast majority of γδ T cells in the blood from all of the cases (**Figure 5**). We verified the existence of all of the major T cell populations defined by the ClusterX clustering algorithm by manually curating several of the original FCS files (**Figures S1B–D**).

The donut plots in **Figure 6** summarize the proportion of $CD69^+$ $HLA\text{-}DR^+$ T cells found in each of the BIL fractions from the 10 surgery cases. At least 90 percent of the T cells isolated from the RE cases and 80 percent of the T cells from the HME case were activated. Even though T cells made up only a small fraction of the $CD45^+$ cells isolated from the TSC and FCD cases, 40–66 percent of the T cells were also activated at the time of surgery.

The absence of $CD8^+$ T_{RM} cells in the BIL fraction from RE case ID 497 suggested that the disease may not have progressed as far in this patient compared with the other two RE cases in the study cohort (Cases IDs 472 and 484). A [^{18}F]-deoxyglucose positron emission tomography (FDG-PET) brain scan of the patient made before the surgery showed hypometabolism extending over the entire affected hemisphere, with no definitive areas of atrophy. In the other two RE cases, areas of atrophy were clearly visible (**Figure 7**). Case ID 497 was the youngest of the three RE patients at the time of seizure onset and underwent surgery after the shortest time following onset of seizures (**Table S1**).

DISCUSSION

We have used CyTOF to gain a better understanding of the involvement of peripheral immune cells in intractable epilepsy in children. We implemented an unsupervised clustering method to resolve the resulting high dimensional data into the main subtypes of adaptive and innate lymphoid cells present in resected epileptogenic brain tissue. Our small cohort study, and other recent work (28) implicate cellular immunity not only in RE, but in intractable epilepsy in children where an infection or immune disorder is not suspected. With the caveat that we only analyzed a limited number of surgery cases, we found a clear difference in the relative number of innate vs. adaptive peripheral immune cells in fractions of $CD45^+$ cells isolated from resected TSC and FCD brain tissue compared with involved RE brain tissue. The FCD and TSC BIL fractions were dominated by a single subset of NK cells (Cluster 8) and a population of myeloid cells (Cluster 1) that are likely to be macrophages, possibly classically activated pro-inflammatory M1 macrophages. Adding CD38 and CD163 antibodies to the staining panel will allow us to distinguish between M1 and M2 macrophages, respectively (29, 30). In an animal seizure model, infiltrating monocytes were shown to exacerbate neuronal damage (31), indicating that the abundant myeloid population that we found in the FCD and TSC BIL fractions may be pathologically relevant.

Most NK cells in the blood express a low level of CD56 ($CD56^{dim}$) and are $CD16^+$, thus can engage in antibody-dependent cell-mediated cytotoxicity by binding antibody-coated target cells via the low affinity Fcγ receptor III (CD16) (32). Fewer circulating NK cells express a high level of CD56 ($CD56^{bright}$); these cells generally lack CD16, but can produce pro-or anti-inflammatory cytokines (32). $CD56^{bright}$ NK cells are considered to be more immature than $CD56^{dim}$ NK cells in that they mostly do not express killer cell immunoglobulin-like receptors (KIRs) (33). Unlike $CD56^{dim}$ NK cells they express CD62L (L-selectin) and CD197 (CCR7), cell surface molecules that facilitate homing to secondary lymph nodes (32). $CD56^{dim}$

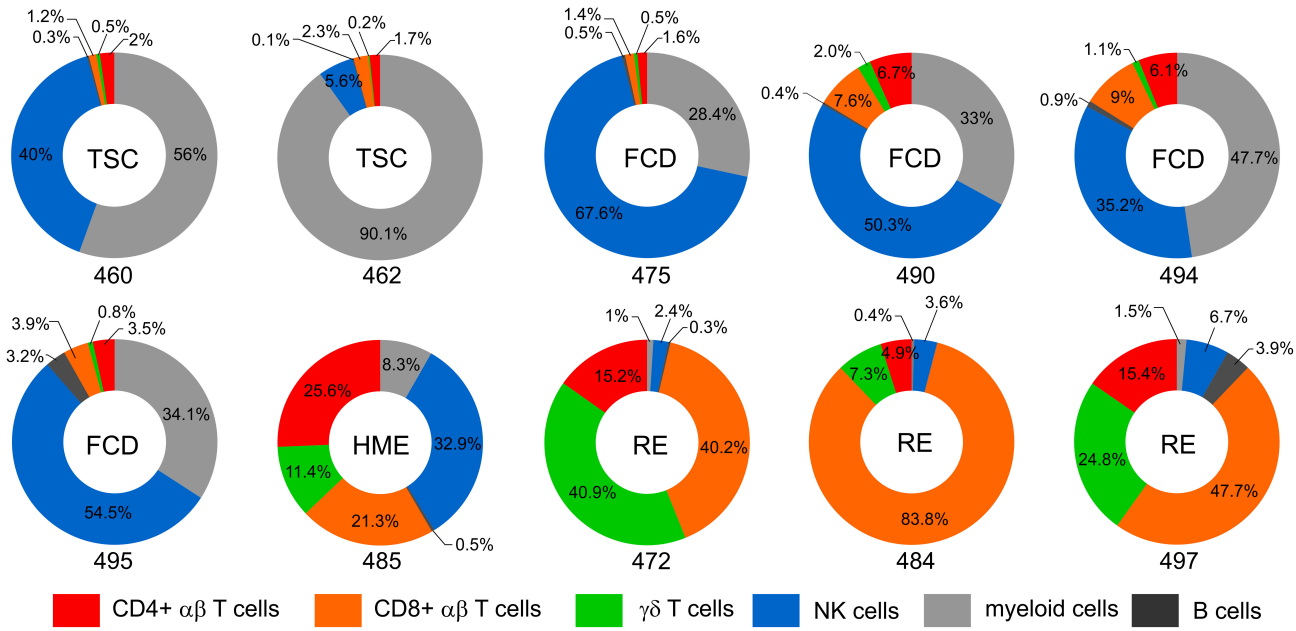

FIGURE 4 | Immune cell profiles of BILs. Donut plots show the relative number of lymphoid cells, myeloid cells and B cells in the BIL fractions isolated from resected brain tissue. Lymphoid cells are divided into CD4, CD8, and γδ T cells and NK cells. Each chart corresponds to one of the surgery cases, and are arranged according to the clinical diagnosis (TSC: 460, 462; FCD: 475, 490, 494, 495; HME: 485; RE: 472, 484, 497). The data are presented as percentages of the number of $CD45^+$ cells in each BIL fraction.

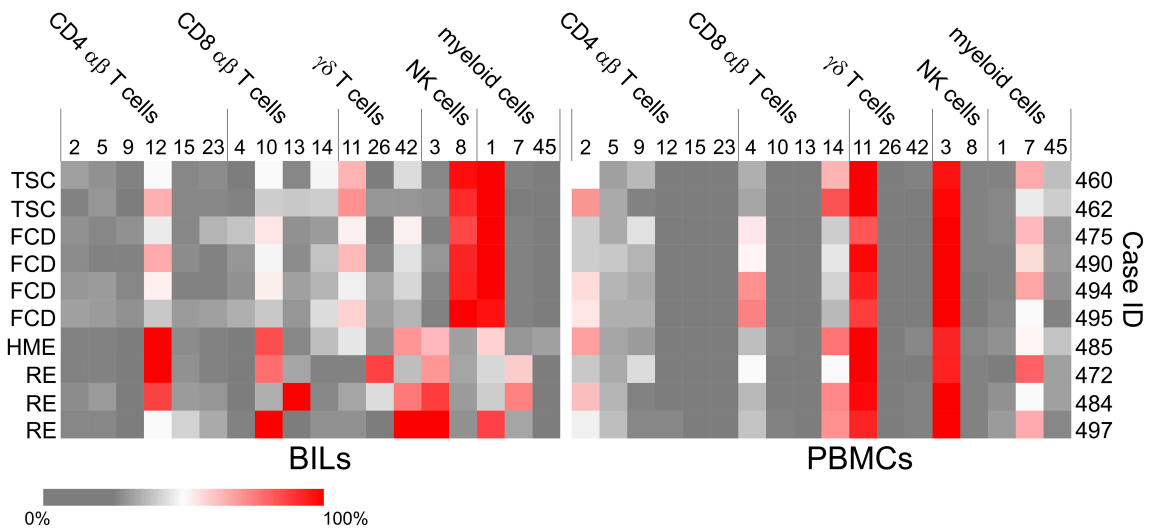

FIGURE 5 | Heat maps showing the subsets of T cells, NK and myeloid cells that account for the majority of lymphoid and myeloid cells in each BILs and PBMC fraction. The heat maps show the relative abundance of each cluster as a percentage of the total number of CD4 αβ T cells, CD8 αβ T cells, γδ T cells, NK cells or myeloid cells, respectively in each BIL and PBMC fraction (**Table S4**).

NK cells express KIRs and CD57 and are considered to be intrinsically more cytotoxic (32, 33). The most abundant NK cells that we found in the blood of all of the patients in our study cohort presumably correspond to $CD56^{dim}CD16^+$ NK cells (Cluster 3). This subset also comprised the majority of NK cells present in the BIL fractions from the RE and HME patients (**Figure 5**). Adding CD62L, CD197, and CD57 to the marker panel will clarify the phenotype of Cluster 3 NK cells.

By comparison to the NK cells in the PBMC fractions, the majority of NK cells found in the FCD and TSC BIL fractions expressed a lower level of CD56 suggesting that they correspond to a $CD56^{dim}$ subset (Cluster 8) (**Figure 2**, **Figure S1A**). Unlike canonical $CD56^{dim}$ NK cells, Cluster 8 cells did not express

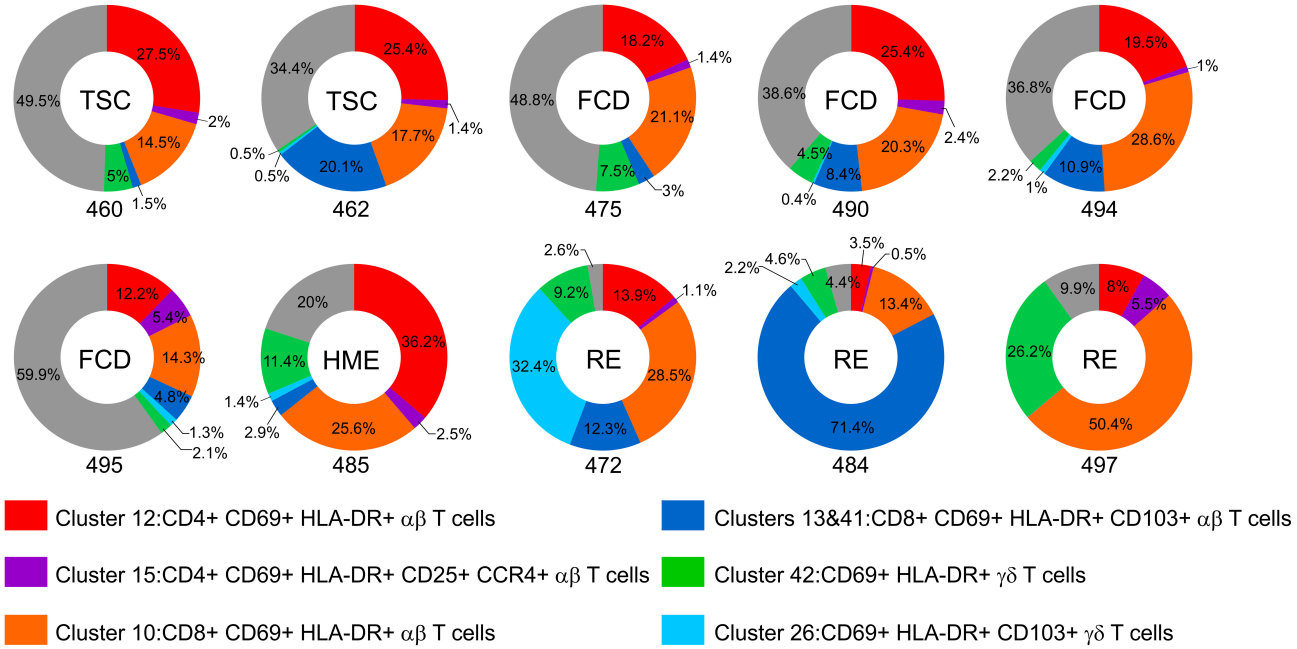

FIGURE 6 | The proportion of activated T cells in the BIL fractions. Donut plots show the relative number of $CD69^{+}$ $HLA\text{-}DR^{+}$ CD4, CD8, and γδ T cells as percentage of the $CD3^{+}$ cells in each BIL fraction. Each chart corresponds to one of the surgery cases, and are arranged according to the clinical diagnosis (TSC: 460, 462; FCD: 475, 490, 494, 495; HME: 485; RE: 472, 484, 497). The different activated subsets are color coded; the gray area denotes the percentage of T cells that did not express activation markers.

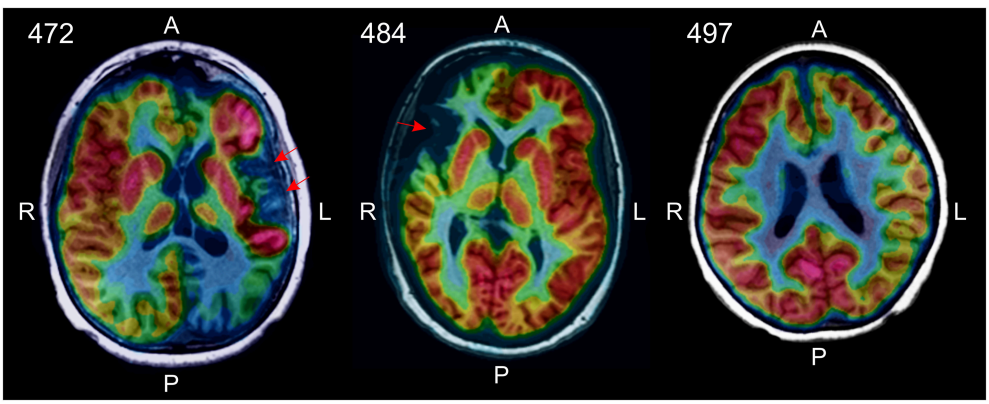

FIGURE 7 | [^{18}F]-deoxyglucose positron emission tomography (FDG-PET) identified areas of atrophy in RE cases 472 and 484 (red arrows). In RE case 497 there were no obvious signs of atrophy in the affected hemisphere, rather decreased glucose metabolism over the entire hemisphere was apparent.

CD16, however populations of $CD56^{dim}$ NK cells expressing little or no CD16 have been previously described (34). Lack of CD16 may be due to the fact that the Cluster 8 NK cells were highly activated; it has been reported that the Fcγ receptor III is proteolytically cleaved in degranulating $CD56^{dim}$ NK cells (35). In contrast to Cluster 3 NK cells, Cluster 8 NK cells also expressed HLA-DR (**Figure 2**, **Figure S1A**), which suggests that they may be adaptive NK cells (32, 33). A third population of NK cells (Cluster 33), only found in appreciable numbers in BILs from the HME case (Case ID 485) expressed CD69 along with CCR5, CXCR3, and CD103 suggesting that this cluster may correspond to an activated resident subset (32).

BIL fractions from the RE cases were predominantly CD8 αβ and γδ effector memory and resident memory T cells with fewer effector memory CD4 T cells, confirming previous work (20, 27). We and others have shown that T cells found in RE brain are clonally focused (7–10), which strongly implicates an antigen-driven adaptive immune response in the etiology of RE, and is consonant with the idea that RE is an autoimmune disease (36). Finding T_{RM} CD8 αβ and γδ T cells in affected RE brain tissue indicates that an earlier immune response had occurred that left

a resident memory population in place. Compared with RE cases 472 and 484 however, no T_{RM} cells were present in the BILs isolated from RE case 497, suggesting that this case may represent an early stage of the disease. In support of this idea, brain atrophy, a feature of late phases of the disease (4), was not evident in an FDG-PET brain scan of RE case 497, but was in RE cases 472 and 484 (**Figure 7**).

In pathological specimens from FCD and TSC surgeries scattered T cells have been previously observed (16, 17), and in our study we found activated CD4, CD8, and γδ T cells in the BIL fractions from the FCD and TSC cases (**Figure 6**). Determining the clonal diversity of these T cells by sequencing Vβ chain third complementarity determining regions may indicate whether they are unspecific bystanders or presage an adaptive response against self-antigens. Binding of self-peptides would however depend on the MHC alleles carried by the individual (37–39). The proportion of activated T cells in the single HME case (Case ID 485), which exceeded the number of NK cells (**Figure 4**), may indicate that an autoimmune response had already occurred in this very young patient who suffered from severe seizures. Future recall assays in which T cells isolated from resected brain tissue are cocultured with autologous neurons and glia differentiated from patient-derived induced pluripotent stems cells will directly answer whether the T cells are autoreactive.

The immune cell profiles of peripheral blood from all of the cases comprised both naïve and antigen experienced T cells. γδ T cells that were $CD127^+$, $CD45RO^+$, $CXCR3^+$, and $CCR5^+$ comprised the majority of γδ T cells in all of the PBMC samples, as well as in the FCD and TSC BILs (Cluster 11; **Figures 2**, **5**, **Figure S1D**). They likely express Vδ2/Vγ9 TCR receptors (40) since Vδ2/Vγ9 γδ T cells make up a large fraction of circulating γδ T cells (41). Expression of CXCR3 and CCR5 would allow these γδ T cells to access sites of inflammation (26). CXCR3 expression by naïve $CD45RA^+$ CD8 T cells (Cluster 4; **Figure 2**, **Figure S1C**) indicates that they are activated (42), and that development into effector T cells may be enhanced (43, 44). Whether the presence of these T cell subsets in the blood is directly related to inflammation in the brain remains to be determined. In future work we plan to compare the immune cell profiles of blood collected at the time of surgery and after recovery from surgery. Removal of epileptogenic brain tissue, the potential source of inflammation may change the relative frequency of specific circulating T cell subsets. Such subsets could potentially be used as biomarkers to assess the extent brain inflammation in children presenting with intractable epilepsy.

The distribution of the two most abundant $CCR4^+$ CD4 T cell subsets (Clusters 5 and 15) differed between the blood and brain. Cluster 15 cells were almost exclusively found in BIL fractions, and appear to be activated effector memory-like Tregs ($CD25^+$ $CD45RO^+CD69^+HLA\text{-}DR^+$) (**Figures 2**, **5**, **Figure S1B**). Tregs are also defined by expression of the transcription factor FOXP3 (24); adding this intracellular marker to the CyTOF panel will confirm the identity of these CD4 T cells. In a cohort of pediatric epilepsy surgery patients, a negative correlation between seizure frequency and the relative number of Tregs in resected epileptogenic brain was recently reported (28). In our study cohort, the relative number of Cluster 15 Tregs cells ranged from 2.61 to 31.53 percent of CD4 T cells in the BIL fractions (**Table S4**). However, the limited number of self-reports of seizure activity available do not allow us to draw any conclusions about the number of seizures and the percent Tregs in our patient cohort. Cluster 5 $CD45RO^+CCR4^+$ CD4 T cells (**Figure 2**, **Figure S1B**), which express both IL-2Rs and IL-7Rs, were found in both BIL and PBMC fractions, and may correspond to a subset of CD4 T cells that express CD25 and CD127 and low levels of FOXP3, and are not suppressive *in vitro* (24). Adding the FOXP3 Ab to the marker panel may help clarify the identity of this subset of CD4 T cells.

Extensive animal studies have shown that inflammation in the brain is both a cause and a consequence of seizure activity (45). Attention has focused on the innate response of brain resident microglia and astrocytes to seizures and on the pro-inflammatory molecules produced by these cells as drivers of epileptogenesis (46–48). In seizure models, chemokines and cytokines produced by activated microglia and astrocytes can cause blood brain barrier leakage, and can attract peripheral lymphoid and myeloid cells to the brain (31, 49–51). Our data suggest that this could be happening in children with chronic pharmacoresistant seizures. Intractable epilepsy might be viewed as a "sterile" infection in which T cells and other immune cells traffic to epileptogenic areas of the cerebral cortex in response to local seizure-driven inflammation. Activated T cells and NK cells for example could permanently alter brain circuitry by directly or indirectly damaging neurons (52–55). Adjunctive treatments that block the recruitment of pro-inflammatory peripheral lymphoid and myeloid cells to the brain (56–61) may therefore be therapeutically beneficial.

AUTHOR CONTRIBUTIONS

GO designed the study, analyzed the data, and wrote the paper. AG assisted with study design and performed the CyTOF. AM assisted with analysis of the data. JC and SR coordinated collection of surgical specimens and prepared BILs and PBMCs. RP assisted with study design and helped draft the paper. NS provided FDG-PET results. GM supported the project. AF provided support for the work, and provided surgical specimens and patient information.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.00121/full#supplementary-material

REFERENCES

1. Guerrini R. Epilepsy in children. *Lancet* (2006) 367:499–524. doi: 10.1016/S0140-6736(06)68182-8
2. Berg AT, Kelly MM. Defining intractability: comparisons among published definitions. *Epilepsia* (2006) 47:431–6. doi: 10.1111/j.1528-1167.2006.00440.x
3. Varadkar S, Bien CG, Kruse CA, Jensen FE, Bauer J, Pardo CA, et al. Rasmussen's encephalitis: clinical features, pathobiology, and treatment advances. *Lancet Neurol.* (2014) 13:195–205. doi: 10.1016/S1474-4422(13)70260-6
4. Bien CG, Urbach H, Deckert M, Schramm J, Wiestler OD, Lassmann H, et al. Diagnosis and staging of Rasmussen's encephalitis by serial MRI and histopathology. *Neurology* (2002) 58:250–7. doi: 10.1212/WNL.58.2.250
5. Prayson RA, Frater JL. Rasmussen encephalitis: a clinicopathologic and immunohistochemical study of seven patients. *Am J Clin Pathol.* (2002) 117:776–82. doi: 10.1309/AD8R-560C-4V11-C5E2
6. Pardo CA, Vining EP, Guo L, Skolasky RL, Carson BS, Freeman JM. The pathology of Rasmussen syndrome: stages of cortical involvement and neuropathological studies in 45 hemispherectomies. *Epilepsia* (2004) 45:516–26. doi: 10.1111/j.0013-9580.2004.33103.x
7. Schwab N, Bien CG, Waschbisch A, Becker A, Vince GH, Dornmair K, et al. CD8+ T-cell clones dominate brain infiltrates in Rasmussen encephalitis and persist in the periphery. *Brain* (2009) 132:1236–46. doi: 10.1093/brain/awp003
8. Schneider-Hohendorf T, Mohan H, Bien CG, Breuer J, Becker A, Gorlich D, et al. CD8(+) T-cell pathogenicity in Rasmussen encephalitis elucidated by large-scale T-cell receptor sequencing. *Nat Commun.* (2016) 7:11153. doi: 10.1038/ncomms11153
9. Dandekar S, Wijesuriya H, Geiger T, Hamm D, Mathern GW, Owens GC. Shared HLA CLASS I and II alleles and clonally restricted public and private brain-infiltrating alphabeta T cells in a cohort of rasmussen encephalitis surgery patients. *Front Immunol.* (2016) 7:608. doi: 10.3389/fimmu.2016.00608
10. Al Nimer F, Jelcic I, Kempf C, Pieper T, Budka H, Sospedra M, et al. Phenotypic and functional complexity of brain-infiltrating T cells in Rasmussen encephalitis. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e419. doi: 10.1212/NXI.0000000000000419
11. Martin KR, Zhou W, Bowman MJ, Shih J, Au KS, Dittenhafer-Reed KE, et al. The genomic landscape of tuberous sclerosis complex. *Nat Commun.* (2017) 8:15816. doi: 10.1038/ncomms15816
12. Polchi A, Magini A, Meo DD, Tancini B, Emiliani C. mTOR signaling and neural stem cells: the tuberous sclerosis complex model. *Int J Mol Sci.* (2017) 19:E1474. doi: 10.3390/ijms19051474
13. Mirzaa GM, Campbell CD, Solovieff N, Goold C, Jansen LA, Menon S, et al. Association of MTOR mutations with developmental brain disorders, including megalencephaly, focal cortical dysplasia, and pigmentary mosaicism. *JAMA Neurol.* (2016) 73:836–45. doi: 10.1001/jamaneurol.2016.0363
14. D'Gama AM, Woodworth MB, Hossain AA, Bizzotto S, Hatem NE, LaCoursiere CM, et al. Somatic mutations activating the mTOR pathway in dorsal telencephalic progenitors cause a continuum of cortical dysplasias. *Cell Rep.* (2017) 21:3754–766. doi: 10.1016/j.celrep.2017.11.106
15. Alcantara D, Timms AE, Gripp K, Baker L, Park K, Collins S, et al. Mutations of AKT3 are associated with a wide spectrum of developmental disorders including extreme megalencephaly. *Brain* (2017) 140:2610–22. doi: 10.1093/brain/awx203
16. Boer K, Jansen F, Nellist M, Redeker S, van den Ouweland AM, Spliet WG, et al. Inflammatory processes in cortical tubers and subependymal giant cell tumors of tuberous sclerosis complex. *Epilepsy Res.* (2008) 78:7–21. doi: 10.1016/j.eplepsyres.2007.10.002
17. Iyer A, Zurolo E, Spliet WG, van Rijen PC, Baayen JC, Gorter JA, et al. Evaluation of the innate and adaptive immunity in type I and type II focal cortical dysplasias. *Epilepsia* (2010) 51:1763–73. doi: 10.1111/j.1528-1167.2010.02547.x
18. Boer K, Crino PB, Gorter JA, Nellist M, Jansen FE, Spliet WG, et al. Gene expression analysis of tuberous sclerosis complex cortical tubers reveals increased expression of adhesion and inflammatory factors. *Brain Pathol.* (2010) 20:704–19. doi: 10.1111/j.1750-3639.2009.00341.x
19. Prabowo AS, Anink JJ, Lammens M, Nellist M, van den Ouweland AM, Adle-Biassette H, et al. Fetal brain lesions in tuberous sclerosis complex: TORC1 activation and inflammation. *Brain Pathol.* (2013) 23:45–59. doi: 10.1111/j.1750-3639.2012.00616.x
20. Owens GC, Erickson KL, Malone CC, Pan C, Huynh MN, Chang JW, et al. Evidence for the involvement of gamma delta T cells in the immune response in Rasmussen encephalitis. *J Neuroinflammation* (2015) 12:134. doi: 10.1186/s12974-015-0352-2
21. Chen TJ, Kotecha N. Cytobank: providing an analytics platform for community cytometry data analysis and collaboration. *Curr Top Microbiol Immunol.* (2014) 377:127–57. doi: 10.1007/82_2014_364
22. Chen H, Lau MC, Wong MT, Newell EW, Poidinger M, Chen, J. Cytofkit: a bioconductor package for an integrated mass cytometry data analysis pipeline. *PLoS Comput Biol.* (2016) 12:e1005112. doi: 10.1371/journal.pcbi.1005112
23. Bien CG, Bauer J, Deckwerth TL, Wiendl H, Deckert M, Wiestler OD, et al. Destruction of neurons by cytotoxic T cells: a new pathogenic mechanism in Rasmussen's encephalitis. *Ann Neurol.* (2002) 51:311–8. doi: 10.1002/ana.10100
24. Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3+ regulatory T cells in the human immune system. *Nat Rev Immunol.* (2010) 10:490–500. doi: 10.1038/nri2785
25. Trebst C, Staugaitis SM, Tucky B, Wei T, Suzuki K, Aldape KD, et al. Chemokine receptors on infiltrating leucocytes in inflammatory pathologies of the central nervous system (CNS). *Neuropathol Appl Neurobiol.* (2003) 29:584–95. doi: 10.1046/j.0305-1846.2003.00507.x
26. Groom JR, Luster AD. CXCR3 in T cell function. *Exp Cell Res.* (2011) 317:620–31. doi: 10.1016/j.yexcr.2010.12.017
27. Owens GC, Chang JW, Huynh MN, Chirwa T, Vinters HV, Mathern GW. Evidence for resident memory T cells in rasmussen encephalitis. *Front Immunol.* (2016) 7:64. doi: 10.3389/fimmu.2016.00064
28. Xu D, Robinson AP, Ishii T, Duncan DS, Alden TD, Goings GE, et al. Peripherally derived T regulatory and gammadelta T cells have opposing roles in the pathogenesis of intractable pediatric epilepsy. *J Exp Med.* (2018) 215:1169–86. doi: 10.1084/jem.20171285
29. Zwadlo G, Voegeli R, Schulze Osthoff K, Sorg C, A monoclonal antibody to a novel differentiation antigen on human macrophages associated with the down-regulatory phase of the inflammatory process. *Exp Cell Biol.* (1987) 55:295–304.
30. Amici SA, Young NA, Narvaez-Miranda J, Jablonski KA, Arcos J, Rosas, L, et al. CD38 is robustly induced in human macrophages and monocytes in inflammatory conditions. *Front Immunol.* (2018) 9:1593. doi: 10.3389/fimmu.2018.01593
31. Varvel NH, Neher JJ, Bosch A, Wang W, Ransohoff RM, Miller RJ, et al. Infiltrating monocytes promote brain inflammation and exacerbate neuronal damage after status epilepticus. *Proc Natl Acad Sci USA.* (2016) 113:E5665–74. doi: 10.1073/pnas.1604263113
32. Cichicki F, Schlums H, Theorell J, Tesi B, Miller JS, Ljunggren HG, et al. Diversification and functional specialization of human NK cell subsets. *Curr Top Microbiol Immunol.* (2016) 395:63–94. doi: 10.1007/82_2015_487
33. Freud AG, Mundy-Bosse BL, Yu J, Caligiuri MA. The broad spectrum of human natural killer cell diversity. *Immunity* (2017) 47:820–33. doi: 10.1016/j.immuni.2017.10.008
34. Amand M, Iserentant G, Poli A, Sleiman M, Fievez V, Sanchez IP, et al. Human CD56(dim)CD16(dim) cells as an individualized natural killer cell subset. *Front Immunol.* (2017) 8:699. doi: 10.3389/fimmu.2017.00699
35. Lajoie L, Congy-Jolivet N, Bolzec A, Gouilleux-Gruart V, Sicard, E, Sung HC, et al. ADAM17-mediated shedding of FcgammaRIIIA on human NK cells: identification of the cleavage site and relationship with activation. *J Immunol.* (2014) 192:741–51. doi: 10.4049/jimmunol.1301024
36. Amrom D, Kinay D, Hart Y, Berkovic SF, Laxer K, Andermann F, et al. Rasmussen encephalitis and comorbid autoimmune diseases: a window into disease mechanism? *Neurology* (2014) 83:1049–55. doi: 10.1212/WNL.0000000000000791
37. Gough SC, Simmonds MJ. The HLA region and autoimmune disease: associations and mechanisms of action. *Curr Genomics* (2007) 8:453–65.
38. Miyadera H, Tokunaga K. Associations of human leukocyte antigens with autoimmune diseases: challenges in identifying the mechanism. *J Hum Genet.* (2015) 60:697–702. doi: 10.1038/jhg.2015.100

39. Gutierrez-Arcelus M, Rich SS, Raychaudhuri S, Autoimmune diseases - connecting risk alleles with molecular traits of the immune system. *Nat Rev Genet*. (2016) 17:160–74. doi: 10.1038/nrg.2015.33
40. Glatzel A, Wesch D, Schiemann F, Brandt E, Janssen O, Kabelitz D. Patterns of chemokine receptor expression on peripheral blood gamma delta T lymphocytes: strong expression of CCR5 is a selective feature of V delta 2/V gamma 9 gamma delta T cells. *J Immunol*. (2002) 168:4920–9. doi: 10.4049/jimmunol.168.10.4920
41. Pang DJ, Neves JF, Sumaria N, Pennington DJ. Understanding the complexity of gammadelta T-cell subsets in mouse and human. *Immunology* (2012) 136:283–90. doi: 10.1111/j.1365-2567.2012.03582.x
42. Groom JR, Luster AD. CXCR3 ligands: redundant, collaborative and antagonistic functions. *Immunol Cell Biol*. (2011) 89:207–15. doi: 10.1038/icb.2010.158
43. Kurachi M, Kurachi J, Suenaga F, Tsukui T, Abe J, Ueha S, et al. Chemokine receptor CXCR3 facilitates CD8(+) T cell differentiation into short-lived effector cells leading to memory degeneration. *J Exp Med*. (2011) 208:1605–20. doi: 10.1084/jem.20102101
44. Groom JR, Richmond J, Murooka TT, Sorensen EW, Sung JH, Bankert K, et al. CXCR3 chemokine receptor-ligand interactions in the lymph node optimize CD4+ T helper 1 cell differentiation. *Immunity* (2012) 37:1091–103. doi: 10.1016/j.immuni.2012.08.016
45. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol*. (2011) 7:31–40. doi: 10.1038/nrneurol.2010.178
46. Vezzani A, Auvin S, Ravizza T, Aronica E. Glia-neuronal interactions in ictogenesis and epileptogenesis: role of inflammatory mediators. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, editors. *Jasper's Basic Mechanisms of the Epilepsies*. Bethesda, MD: Oxford University Press (2012).
47. Devinsky O, Vezzani A, Najjar S, De Lanerolle NC, Rogawski MA. Glia and epilepsy: excitability and inflammation. *Trends Neurosci*. (2013) 36:174–84. doi: 10.1016/j.tins.2012.11.008
48. Eyo UB, Murugan M, Wu LJ. Microglia-neuron communication in epilepsy. *Glia* (2017) 65:5–18. doi: 10.1002/glia.23006
49. Vezzani A, Ravizza T, Balosso S, Aronica E. Glia as a source of cytokines: implications for neuronal excitability and survival. *Epilepsia* (2008) 49 (Suppl. 2):24–32. doi: 10.1111/j.1528-1167.2008.01490.x
50. Fabene PF, Bramanti P, Constantin G. The emerging role for chemokines in epilepsy. *J Neuroimmunol*. (2010) 224:22–7. doi: 10.1016/j.jneuroim.2010.05.016
51. Fabene PF, Laudanna C, Constantin G. Leukocyte trafficking mechanisms in epilepsy. *Mol Immunol*. (2013) 55:100–4. doi: 10.1016/j.molimm.2012.12.009
52. Melzer N, Meuth SG, Wiendl H. CD8+ T cells and neuronal damage: direct and collateral mechanisms of cytotoxicity and impaired electrical excitability. *Faseb J*. (2009) 23:3659–73. doi: 10.1096/fj.09-136200
53. Kreutzfeldt M, Bergthaler A, Fernandez M, Bruck W, Steinbach K, Vorm M, et al. Neuroprotective intervention by interferon-gamma blockade prevents CD8+ T cell-mediated dendrite and synapse loss. *J Exp Med*. (2013) 210:2087–103. doi: 10.1084/jem.20122143
54. Ehling P, Melzer N, Budde T, Meuth SG. CD8(+) T cell-mediated neuronal dysfunction and degeneration in limbic encephalitis. *Front Neurol*. (2015) 6:163. doi: 10.3389/fneur.2015.00163
55. Di Liberto G, Pantelyushin S, Kreutzfeldt M, Page N, Musardo S, Coras R, et al. Neurons under T cell attack coordinate phagocyte-mediated synaptic stripping. *Cell* (2018) 175:458–471 e19. doi: 10.1016/j.cell.2018.07.049
56. Sotgiu S, Murrighile MR, Constantin G. Treatment of refractory epilepsy with natalizumab in a patient with multiple sclerosis. Case report. *BMC Neurol*. (2010) 10:84. doi: 10.1186/1471-2377-10-84
57. Bittner S, Simon OJ, Gobel K, Bien CG, Meuth SG, Wiendl H. Rasmussen encephalitis treated with natalizumab. *Neurology* (2013) 81:395–7. doi: 10.1212/WNL.0b013e31829c5ceb
58. Martin-Blondel G, Brassat D, Bauer J, Lassmann H, Liblau RS. CCR5 blockade for neuroinflammatory diseases–beyond control of HIV. *Nat Rev Neurol*. (2016) 12:95–105. doi: 10.1038/nrneurol.2015.248
59. Ayzenberg I, Hoepner R, Kleiter I. Fingolimod for multiple sclerosis and emerging indications: appropriate patient selection, safety precautions, and special considerations. *Ther Clin Risk Manag*. (2016) 12:261–72. doi: 10.2147/TCRM.S65558
60. Robert R, Juglair L, Lim EX, Ang C, Wang JH, Ebert G, et al. A fully humanized IgG-like bispecific antibody for effective dual targeting of CXCR3 and CCR6. *PLoS ONE* (2017) 12:e0184278. doi: 10.1371/journal.pone.0184278
61. Liang F, Giordano C, Shang D, Li Q, Petrof BJ. The dual CCR2/CCR5 chemokine receptor antagonist Cenicriviroc reduces macrophage infiltration and disease severity in Duchenne muscular dystrophy (Dmdmdx-4Cv) mice. *PLoS ONE* (2018) 13:e0194421. doi: 10.1371/journal.pone.0194421

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Autoimmunity in Parkinson's Disease: The Role of α-Synuclein-Specific T Cells

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Evidence from a variety of studies implicates a role for the adaptive immune system in Parkinson's disease (PD). Similar to multiple sclerosis (MS) patients who display a high number of T cells in the brain attacking oligodendrocytes, PD patients show higher numbers of T cells in the ventral midbrain than healthy, age-matched controls. Mouse models of the disease also show the presence of T cells in the brain. The role of these infiltrating T cells in the propagation of disease is controversial; however, recent studies indicate that they may be autoreactive in nature, recognizing disease-altered self-proteins as foreign antigens. T cells of PD patients can generate an autoimmune response to α-synuclein, a protein that is aggregated in PD. α-Synuclein and other proteins are post-translationally modified in an environment in which protein processing is altered, possibly leading to the generation of neo-epitopes, or self-peptides that have not been identified by the host immune system as non-foreign. Infiltrating T cells may also be responding to such modified proteins. Genome-wide association studies (GWAS) have shown associations of PD with haplotypes of major histocompatibility complex (MHC) class II genes, and a polymorphism in a non-coding region that may increase MHC class II in PD patients. We speculate that the inflammation observed in PD may play both pathogenic and protective roles. Future studies on the adaptive immune system in neurodegenerative disorders may elucidate steps in disease pathogenesis and assist with the development of both biomarkers and treatments.

Keywords: Parkinson's disease, T cells, adaptive immune system, autoimmunity, α-synuclein, neuroinflammation, blood-brain barrier

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INTRODUCTION: PARKINSON'S DISEASE AND INFLAMMATION

Multiple studies have highlighted an association between sustained inflammation and a number of neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), amyotropic lateral sclerosis (ALS), and frontal temporal dementia (FTD) (1). The role of inflammation in the pathogenesis of these disorders, however, remains undetermined. Here, we focus on the inflammatory features in PD, the most common movement disorder, affecting

more than 10 million people worldwide (2). PD patients manifest motor symptoms including bradykinesia, rest tremor, muscular rigidity, and postural and gait impairment, as well as non-motor symptoms (3). Non-motor symptoms include mood disorders, cognitive impairments, and autonomic dysfunction, such as orthostatic hypotension and constipation (3). While alleles of many genes are associated with the disorder, PD remains largely (∼90% of cases) a sporadic disorder associated with older age and various genetic and environmental risk factors (4).

PD is diagnosed from motor symptoms (3), but non-motor symptoms are often manifest during a prolonged prodromal phase as much as 20 years prior to the onset of the motor features (5). These prodromal non-motor symptoms include constipation, rapid eye movement (REM) sleep behavior disorder, depression, anosmia, and excessive daytime sleepiness (6). The sensitivity and the specificity of these non-motor symptoms limits their utility in predicting the development of PD (6).

Pathologically, PD brains are characterized by progressive degeneration of dopaminergic neurons in the substantia nigra (SN) pars compacta and Lewy pathology. Misfolded α-synuclein (α-syn) becomes insoluble and aggregates to form intracellular inclusions within the neuronal cell bodies (Lewy bodies) and processes (Lewy neurites) (7). Lewy pathology is not only restricted to the central nervous system (CNS) but is also present in the peripheral nervous system (PNS), cardiac plexus, sympathetic ganglia, enteric nervous system (ENS), and other neurons (8). α-Syn aggregates are hypothesized to progress from the periphery, through the spinal cord, into the midbrain and from there across multiple cortical brain regions, as classified in Heiko Braak's staging system (8).

Also noted but often overlooked is the degree of inflammation in PD, both in the periphery and in the brain. Autoreactive T lymphocytes, autoantigen presentation, and microglial activation are detectable in PD patients and animal models of the disease. The recent identification of α-syn-specific T cells in PD patients compounded with α-syn pathology and misprocessing provides a basis for the hypothesis that PD may have features of an autoimmune disorder. It is possible that inflammatory characteristics during and after the prodromal phase and disease might be related. These features, including the adaptive immune response, could play a role in PD pathogenesis or be of diagnostic value even during the prodromal phase.

PATIENT DATA: PERIPHERAL PATHOLOGY, CHANGES IN T CELL SUBPOPULATIONS, LYMPHOCYTE INFILTRATION INTO THE CNS AND THEIR ROLES IN PD ETIOLOGY

Peripheral Pathology

PD patients display symptoms of gut dysmotility and altered microbial composition in the intestine, but the cause of these disruptions and their role in pathogenesis remain unclear. Constipation is often an early symptom of PD, preceding motor symptoms by more than 10 years (9, 10) and worsens with disease progression (11). Clinical studies have revealed altered gut microbiota composition in PD patients that may either increase inflammation (12, 13) or gut permeability (14, 15). Indeed, higher intestinal permeability has been observed early in PD pathogenesis (16, 17) and has been associated with enteric α-syn pathology (18). A leaky intestine permits the translocation of the microbes and their products, such as LPS, which initiate sustained inflammation, as well as cells involved in inflammatory responses. It is possible that sustained inflammation can drive α-syn deposition into the ENS. Chen et al. demonstrated that aged Fisher rats fed amyloid-protein curli-producing *Escherichia coli* display increased neuronal expression of α-syn in the submucosal and myenteric plexus of the gut as well as in the brain (19). Possible effects of altered microbiota in PD were illustrated in an α-syn transgenic mouse of PD (20). Transgenic mice grown in germ-free environments exhibited milder symptoms than mice with regular gut microbiota (20). In addition, germ-free mice that were transplanted with PD patient microbiomes displayed worsened motor dysfunction (20).

Influential studies by Braak et al. identified the dorsal motor nucleus of the vagus (DMV) and the ENS of PD patients as early locations for Lewy pathology prior to the *substantia nigra* (8, 21, 22). They hypothesize that α-syn deposition begins in the gut and travels through the vagus nerve into the CNS (8). α-Syn labeling in nerve fibers of the colon is observed in early stage untreated PD patients but is absent in healthy controls or irritable bowel syndrome patients (23), although these findings have not been confirmed in large autopsy cohorts (24, 25).

The chronology of prodromal symptoms has been investigated in a rotenone mouse model of PD. Exposure to rotenone, a pesticide that inhibits complex I of the mitochondrial respiratory chain (26), is linked to PD (27). Chronic, intragastric administration of low doses of rotenone to mice for 1.5 months causes α-syn aggregation in the ENS, DMV, and intermediolateral nucleus of the spinal cord without motor dysfunction (28). Gut motility impairments are observed after 2 months of rotenone treatment (29). After 3 months, α-syn aggregation and loss of dopaminergic neurons is observed in the SN (28). Moreover, α-syn released by enteric neurons may be taken up by presynaptic sympathetic neurites and retrogradely transported to the soma in this model (29). The intragastric rotenone model of PD has been claimed to accurately recapitulate the spatiotemporal development of pathological and clinical symptoms and supports the Braak hypothesis that α-syn pathology begins in the periphery and retrogradely ascends the CNS (8).

Gut pathology is also linked to intestinal inflammation in PD patients. Increased levels of pro-inflammatory cytokines, such as TNFα (tumor necrosis factor α), interleukin (IL)-1β, IL-6, and IFNγ (interferon-γ), are observed and are negatively correlated with disease duration (30). In addition, $CD4^+$ T cells infiltrate the colonic mucosa of PD patients with constipation at higher numbers than in PD patients without constipation (31). The gut may be an initiating site of inflammation and pathology and could be the location in which the adaptive immune system is primed against α-syn deposition.

Changes in T Cell Subpopulations and Cytokines

Consistent with the "systemic" view that PD involves multiple systems and tissues, several studies have shown general alterations in cytokines and immune cell populations.

Proinflammatory cytokines are elevated in the blood of PD patients, including increased levels of IL-2 (32, 33)—6 (34–38)—8 (38), MCP-1 (monocyte chemoattractant protein-1) (38), MIP-1α (macrophage inflammatory protein-1 α) (38), RANTES (regulated upon activation, normal T-cell expressed and secreted) (38, 39), TNFα (35, 36, 40, 41), and IFNγ (38). Increased levels of proinflammatory cytokines and chemokines are indicative of an immune system responding to tissue damage and/or foreign molecules. The levels of cytokines and chemokines correlate with the clinical stage of the disease, highlighting a role for peripheral inflammation in PD progression (38). Altered T cells populations can also contribute to the changes in circulating cytokines. Th1 and TH17$^+$ CD4$^+$ cells can contribute to the increased levels of IFNγ, TNFα, and IL-17 (42, 43).

Several studies have compared the T cell subpopulations in PD patients with those of healthy controls. PD patients are found to have fewer naïve T cells (44, 45), including CD4$^+$ Th (helper) cells (44, 46, 47), and more activated T cells (48), including Th2 regulatory T cells (Tregs) (44, 46), than healthy age-matched controls. Some studies have reported a general decrease in total Th cells (46, 49, 50), Tregs (42, 51), and CD8$^+$ cytotoxic T cells (50), whereas other studies show no change in Treg number (52) with an increase in Th1 cells, but more significantly the Th17 subset in PD patients (43, 51, 53). These disparate findings in the levels of naïve, helper, cytotoxic, and regulatory T cells in PD may be due to differences in phenotyping methods and patient recruitment. Note further that these studies only examine the total bulk populations of T cells without discerning changes potentially related to autoantigen recognition, which may represent a very small fraction of the total populations. It will be important to reexamine these results in light of the recent growing definition of the antigen specificities of T cells associated with neurodegenerative diseases.

The number and proportion of T cells types provide clues to their roles. Tregs respond to heightened inflammation by suppressing effector T cell proliferation and cytokine production (54) and their dysregulation can lead to autoimmune disorders. PD patient-derived Tregs have been shown to have an impaired ability in suppressing effector T cell proliferation *in vitro* compared to Tregs of healthy controls (45). In addition, effector T cells from PD patients produce higher amounts of proinflammatory cytokines in response to T cell activators *in vitro* and their Tregs fail to suppress cytokine release (42). If Tregs are dysfunctional in PD, the heightened and persistent proinflammatory environment observed in patients may remain unabated throughout the nervous system and periphery.

Blood-Brain Barrier Breakdown

Increased levels of peripheral cytokines act on CNS endothelial cells that form the blood-brain barrier (BBB) to increase vascular permeability (55). The CNS was formerly considered an immunologically privileged site, largely due to the presence of the BBB, a physical barrier formed by endothelial cells via interactions with pericytes and astrocytes to prevent blood proteins, antibodies, immune cells, and drugs from penetrating into the brain parenchyma (56). The BBB, however, becomes disrupted during acute and chronic inflammation. Under sustained chronic inflammation, tight junctions between endothelial cells that prevent paracellular diffusion are weakened or disrupted, allowing the passage of antibodies or immune cells that would otherwise be restricted from the brain (57). In addition, inflamed CNS endothelial cells upregulate expression of adhesion molecules (e.g., ICAM and VCAM-1) that bind and recruit circulating T cells and monocytes, as well as proteins (e.g., caveolin-1, PLVAP) that promote transcellular transport of immune cells and antibodies across the barrier (55, 58). A compromised BBB renders the brain susceptible to circulating immune cells, antibodies, and pro-inflammatory cytokines. T cells can then extravasate into the brain via the disrupted BBB, as seen in both MS patients and animal models for the disease (55, 58).

Neuroimaging studies on PD patients show that the BBB is compromised in the vicinity of the midbrain (59) as well as various deep and cortical gray matter and white matter regions (60, 61). In post-mortem tissue, there is evidence of brain capillary leakage indicated by deposition of blood-derived proteins (e.g., fibrinogen and IgG) in the striatum and subthalamic nucleus (62, 63). Capillary leakage associated with microvascular degeneration, disrupted tight junctions, changes in capillary basement membrane composition in the subthalamic nucleus (63), and aberrant angiogenesis (marked by increased levels of integrin αvβ3, a proangiogenic integrin expressed by immature endothelial cells) have been found within the SN, locus coeruleus, and putamen of PD brains (64).

LYMPHOCYTE INFILTRATION AND ITS ROLE IN PD ETIOLOGY

T Cells in the Midbrain of PD Patients

Perhaps as a result of peripheral inflammation, changes in lymphocyte subtype populations, and BBB breakdown, T cells can infiltrate the affected brain regions of PD patients. First reported by the McGeers in 1988, CD3$^+$ cells, a marker for T cells, were detected within the CNS of PD brains (65, 66). Brochard et al. showed that both CD4$^+$ and CD8$^+$ T cells, but not B and natural killer (NK) cells, infiltrated the SN of PD patients and were present at much far greater levels than in healthy controls. These T cells were located near blood vessels and neuromelanin-containing dopaminergic neurons (43, 67). The presence of T cells in the region affected in the disease suggests a targeted extravasation, rather than a random consequence of increased BBB permeability by peripheral inflammation. If T cells that had escaped self-tolerance circulate in the blood of PD patients, it is plausible that they could infiltrate into the brain permitted by a leaky BBB. The causal role of infiltrating T cells is further elucidated in studies from mouse models of PD.

Data From Animal Models Support a Role for T Cells in Disease Pathogenesis

Although animal models have limitations in recapitulating PD, they are useful tools for genetic manipulation and identifying features of disease pathology. A range of models for studying PD have been developed, employing toxins or genetic mutations that recapitulate certain aspects of the disease. Most of the models highlight a causative role for infiltrating T cells in propagating neurodegeneration.

Toxin Models of PD

Intracerebral injections of the toxin 6-hydroxy-dopamine (6-OH-DA) into the midbrain of mice induces degeneration of dopamine and noradrenaline neurons (68), which are rendered vulnerable to the drug because they express the dopamine transporter, DAT, that accumulates the toxin. Once in the neuronal cytosol, 6-OHDA mediates oxidative stress-related cytotoxicity (69–71). The subsequent acute neurodegeneration is manifested in motor deficits in mice and, if injected unilaterally, in rotational motions. 6-OHDA treated mice show IgG leakage, indicative of a leaky BBB, as well as T and B cell infiltration around CNS blood vessels (72). Treg cells are significantly decreased in the periphery of 6-OHDA treated rats, which are reported to not show marked T cell infiltration into the midbrain (73).

Another toxin model uses 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), originally identified as a contaminant in intravenous drug users who developed PD (74, 75). MPTP is lipophilic and readily crosses the BBB and it is then internalized by astrocytes where it is metabolized to MPP^+. Astrocytes release MPP^+, which is taken up by dopaminergic neurons via DAT (76, 77). Once accumulated, MPP^+ induces SN death by inhibiting complex I in the electron transport chain, depleting cells of ATP, and increasing oxidative stress in a dopamine and calcium-dependent manner (78–81).

T cells extravasate into the SN as soon as 2 days post MPTP treatment (82, 83), following microgliosis (67). A causative role for T cells in MPTP-induced dopaminergic degeneration was investigated using immunodeficient mouse strains. Mice that lack functioning T cells (*Rag1*$^{-/-}$ and *Tcrb*$^{-/-}$ mice) are protected from MPTP-induced neurodegeneration. Despite the observation that $CD8^+$ T cells are more numerous in the midbrain following MPTP, *Cd8a*$^{-/-}$ mice were not protected from neuronal death. Mice that lack functioning $CD4^+$ (*Cd4*$^{-/-}$ mice) cells, however, were protected from loss of dopaminergic neurons (67), implicating this arm of the adaptive immune system. SCID mice, which are deficient in functional T and B cells, were also protected against MPTP induced neuronal death (84). Neurodegeneration was restored in SCID mice that are reconstituted with wild-type splenocytes (84). These studies reinforce the notion that a functioning immune system, and $CD4^+$ T cells in particular, may be essential for neurodegeneration in the MPTP model of PD.

Interestingly, dopamine (DA) receptors are expressed by cells of the innate and adaptive immune response, including $CD8^+$ and $CD4^+$ T cells. Expression of the D3-type receptor (D3R), one of the five DA receptors in humans, was significantly reduced in PD patient T cells and is correlated with disease severity (85). D3R-deficient mice treated with MPTP were protected from dopaminergic neuron death and microglial activation, but the protection was lost with the transfer of $CD4^+$ T cells from wild-type animals (86). Additionally, *Rag1*$^{-/-}$ mice that were protected against MPTP become susceptible to neurodegeneration with the transfer of WT $CD4^+$ T cells but not when reconstituted with D3R-deficient $CD4^+$ T cells (86). These data suggest that D3R signaling in T cells may be necessary in the MPTP mouse model.

T cell receptor stimulation is reported to induce D3R expression on $CD4^+$ T cells, and D3R signaling on $CD4^+$ T cells contributes to the expansion of Th1 and Th17 cells in the context of inflammatory colitis (87). DA is in part depleted in PD, as a subset of dopaminergic and norepinephrinergic neurons are lost in the disease. The role of DA receptors on T cells could evolve over the disease course, including via decreased activation due to DA loss and spikes of activation with treatment of DA replacing drugs. The level and activation profile of these receptors throughout the disease could shed light on their role in contributing to a proinflammatory T cell profile, particularly during drug treatment.

While $CD8^+$ and $CD4^+$ Th1 T cells can exert cytotoxic and proinflammatory effects, $CD4^+$ Tregs exert anti-inflammatory properties that protect cells against neurotoxic molecules or persistent inflammation. Adoptive transfer of Th cells, induced by co-polymer immunization, into MPTP mice protects dopaminergic neurons. This protection seems to be mediated via suppression of microglial activation (88–91).

Viral Overexpression of α-Syn to Model PD

A different approach to modeling PD uses targeted overexpression of human α-syn in the midbrain of rodents. In one model, α-syn is overexpressed in the SN by injection with recombinant adeno-associated virus vector (AAV2-SYN), which leads to the slow degeneration of dopaminergic neurons (92). Consistent with findings from the toxin mouse models above, AAV2-SYN injected mice display IgG deposition as a consequence of BBB breakdown, as well as T and B cell infiltration (92). The AAV2-SYN model induces a slower, less acute neurodegeneration than the toxin-based PD approaches, and so may prove useful for understanding the timing of inflammation and neuronal death. Importantly, human α-syn overexpression triggers microglial activation, BBB leakage, and recruitment of T and B cells into the SN *prior* to neurodegeneration in mice (92). These findings support the concept that immune cell recruitment and local inflammatory features play roles in neurodegeneration, rather than that they are consequences downstream from neuronal death.

In a rat model of PD, overexpression of a rare, autosomal dominant A53T mutant α-syn allele that causes α-syn aggregation and early onset PD in humans, induces microgliosis and lymphocytic infiltration in SN (93). However, administration of FK506, an immunosuppressive drug that inhibits T cell signal transduction and IL-2

transcription, on the rAAv2/7 α-syn overexpressing rat model increased the survival of dopaminergic neurons, with a positive trend for motor improvement. FK506 reduced the numbers of microglia, macrophages, and $CD4^+$ T cells in the SN and delayed the infiltration of $CD8^+$ T cells (93).

In vitro Studies of iPSC-Derived Dopamine Neurons With Th17 Cells

A recent *in vitro* study using induced-pluripotent stem cells (iPSC)-derived midbrain neurons and T cells from PD patients was the first to show that PD patient-derived T cells can kill dopamine neurons directly. Sommer et al. determined that PD patients contain significantly higher Th17 cells than healthy controls (43). The PD patient-derived Th17 cells exerted cytotoxic effects on neurons by releasing IL-17A, a cytokine detected by IL-17R expressed on neurons (43). The iPSC *in vitro* cultures lacked glia, which express MHC-II and can potentially interact with Th17 cells. In addition, T cells were activated non-specifically, and so the antigen specificity of Th17 cells remains unclear (43). While the study indicates that PD-derived T cells can directly kill dopaminergic neurons, the omission of professional antigen presenting cells, antigenicity, and neuronal specificity in the cultures in this initial study overlooks the role of multiple relevant *in vivo* factors important for disease progression. Moreover, the mode of action that garners specific vulnerability of dopaminergic neurons and avoids unaffected neurons was not resolved in this study. Nevertheless, the study indicates Th17 cells may participate in PD-related neuronal death.

Th17 cells play a prominent role in multiple sclerosis (MS), a well-characterized autoimmune neurological disease. MS patients suffer from white and gray matter lesions of neuronal demyelination and axonal loss due to a dynamic interplay between the adaptive immune system, glia, and neurons (94). The origin of the primary lesion and subsequent inflammation is heavily debated; however, studies in patients and animal models highlight a critical role for T and B cells in MS pathogenesis (95). Increased serum levels of IL-17A and Th17 cells in MS patients has been reported, ranging from 0.77 to 2.48 and 0.34 to 1.55% of total $CD4^+$ T cells in MS patients compared to healthy controls, respectively (96). These levels are higher than those detected in PD patients (43). In the experimental autoimmune encephalomyelitis (EAE) model of MS, myelin oligodendrocyte glycoprotein-specific Th17 cells form direct contacts with neuronal cells in demyelinating lesions and are associated with extensive axonal damage (97). Th17 cells seem to exert their neurotoxic effects by releasing IL-17A, which interferes with the remyelination process (98) and directly damages the BBB (99). Th17 cells also release GM-CSF, which is essential for inducing EAE (100, 101) and likely supports the recruitment of peripheral monocytes (100, 102), and IFNγ that activate infiltrating macrophages (103). Interestingly, studies on the mode of action of Th17 cells in EAE do not include IL17R mediated neurotoxicity. Studies on the role of Th17 in MS patients and mouse models (EAE), could be emulated in PD mouse models to further determine the role and mode of action of Th17 lymphocytes in disease pathogenesis.

The Role of MHC Proteins in PD Pathogenesis

T cells recognize a complex formed between MHC molecules (known as HLA; human leukocyte antigen in humans) and a peptide epitope. The epitope is presented to T cells as a result of a complex series of events termed antigen processing and presentation that involve the intracellular degradation of self and foreign proteins into peptides that are then loaded onto the antigen-binding groove of MHC molecules. In general, T cells recognize peptides derived from foreign molecules, but in disease some T cells also evade self-tolerance and can recognize self-peptides. MHC class I in general presents peptides derived from the same cell (endogenous presentation), while MHC class II typically present peptides derived from extracellular proteins (exogenous presentation).

Mature neurons are generally thought to lack MHC expression and so to not present antigens. However, SN and LC neurons recently have been shown to express MHC-I and co-localize with $CD8^+$ T cells in response to stimuli such as IFNγ (104). Catecholamine neurons are particularly sensitive to inflammation in comparison to other neuron types. A lower amount of IFNγ is required by dopaminergic neurons to express MHC-I in comparison to other neuronal subtypes. The MHC-I presented by dopaminergic neurons is functional and can result in cytotoxicity in the presence of matching antigen and $CD8^+$ T cells (104). Dopaminergic neuron sensitivity to inflammation and corresponding MHC-I upregulation could render them susceptible to infiltrating T cells versus neighboring, more resistant GABAergic neurons.

GWAS in PD have shown an association with haplotypes of MHC II genes, including DRB5*01 and DRB1*15:01 alleles and a polymorphism in a non-coding region that may increase levels of MHC class II expression (105–109). MHC-II positive microglia are detected in the SN of PD patients (65) and their levels increase with disease severity (110).

Animal models of PD have been employed to investigate the role of MHC in neurodegeneration. AAV2- α-syn overexpression in mice that lack MHC-II are protected against microglial activation, BBB permeability, and neuronal death in the SN (111). A necessity for MHC-II signaling for riving neuronal death is reported in the MPTP model of PD. MHC-II null mice are resistant to neuronal death by MPTP intoxication and their microglia do not release pro-inflammatory cytokines in contrast to wild-type animals (112).

Association of HLA alleles to late-onset PD and a positive correlation between levels of MHC-II expression and disease severity in humans, in conjunction with the necessity for MHC-II expression in PD mouse models, suggests that antigen presentation and adaptive immune signaling may be involved in dopaminergic neuron death. Infiltrating T cells, MHC expression, and sensitivity of dopaminergic neurons to inflammation support the concept that PD possesses features of an autoimmune disorder.

The Connection With Innate Immunity and Antigen Presenting Cells

While functioning T cells and MHC signaling may be critical in modeling PD pathology, activated peripheral antigen presenting cells and microglia are potential initiators and drivers of neuroinflammation. Professional antigen presenting cells detect foreign pathogens via pattern recognition receptors (PRR), such as toll-like receptors (TLRs), activating signaling cascades that change the inflammatory profile of the cell (113). Picomolar concentrations of α-syn, possibly relevant to extracellular levels in PD, sensitized the response by macrophages to release proinflammatory cytokines (114). Extracellular neuromelanin derived from human autopsy also activates microglia via CD11b/CD18 (also known as Mac-1) integrin receptors (115). Thus, it is not surprising that peripheral blood monocytes isolated from PD patients have been shown to be hypersensitive and predisposed to an inflammatory stimulus (116). Antigen presenting cells in the periphery have the potential to be the first in line to respond to sustained, slight increases in α-syn levels, which have deleterious effects over time. In addition, *in vitro* studies have shown that α-syn acts as a chemoattractant to neutrophils and monocytes (117). Peripherally activated antigen presenting cells such as monocytes can extravasate into different tissues, including the brain, during active disease states (118). In an AAV overexpressing model of PD, α-syn expression recruited peripheral monocytes to the brain and their recruitment was necessary for dopaminergic neuron death (119). α-Syn in the periphery and CNS acts as an activator and recruiter of professional antigen presenting cells, potentially initiating an inflammatory response against itself.

Microglia are the primary immune cells of the CNS and act as both resident phagocytic and antigen presenting cell to provide an active immune defense (120). Microgliosis is characterized by microglia proliferation, change in morphological state from ramified to amoebic, and the presence of several inflammatory markers, such as CD68 (120). Microglial activation is an important potential player in PD-associated inflammation and immune reactivity. Neurons do not express Class II, while microglia are MHC Class II expressing antigen-presenting cells. MHC Class II present peptides derived from extracellular proteins (exogenous presentation), and cells of the monocyte lineage such as microglia are particularly apt at internalizing aggregated proteins (e.g., α-syn aggregates) and presenting peptides derived from exogenous proteins in the context of class II MHC molecules. They therefore represent a strong candidate for MHC class II restricted presentation of α-syn peptides to T cells.

In vivo imaging studies of microglial activation using positron-emission tomography (PET) showed that PD patients have markedly increased neuroinflammation in various brain regions, including the basal ganglia and striatum, regardless of the number of years with disease (121, 122). Studies have determined that inhibiting or preventing microglial activation renders PD mouse models resistant to neuronal death. RANTES and eotaxin, two chemokines secreted by activated microglia, are upregulated in MPTP mice and monkeys (123, 124). Treatment of MPTP monkeys with NEMO-binding domain (NBD) peptide, which selectively inhibits NF-κB activation, decreases microglia expression of RANTES and attenuates $CD8^+$ T cell infiltration into the SN (123). Neutralization of RANTES and eotaxin in MPTP-intoxicated mice decreases lymphocyte infiltration in the SN, reduces inflammation, and confers dopaminergic neuronal protection and improved motor function (124). The neuron-derived chemokine fractalkine (CX3CL1) is a ligand for CX3CR1 on microglia and promotes sustained microglial activation. Mice that are CX3CR1-deficient are protected against dopaminergic neuron loss in MPTP-treated mice (125). CX3CR1 in microglia are likely limiting the neurotoxic effect of CCL2 induction by astrocytes, which promotes $CCR2^+$ monocyte infiltration (126). Treatment of 6-OHDA rats with safinamide suppressed microglial activation and protected dopaminergic neurons from degeneration (127). In addition, deficits in microglial restraint exacerbate dopaminergic neurodegeneration in 6-OHDA rats (128). Taken together, these studies show that microglial activation is critical for immune cell recruitment and neuronal death in PD. α-Syn itself may activate microglia and antigen presenting cells, initiating an immune response in the brain and periphery. Peripheral α-syn-specific T cells may migrate to the brain and be further activated by α-syn-presenting microglia that have internalized aggregates.

A Model of α-Syn Recognition in PD

It is possible that the innate immune system in the SN is activated and processes and presents α-syn aggregates to specific T cells in the brain region prior to neuronal death. While it is unclear which factors activate microglia, numerous *in vitro* studies show that α-syn or conditioned media from α-syn expressing neurons robustly activate microglia. Treatment of primary microglia with recombinant, monomeric α-syn consistently induces an activated, pro-inflammatory profile, such as increased TNFα, IL-1β, IL-6, COX-2 expression, and iNOS production. When α-syn is overexpressed in mouse models, either via virus injection or transgenically, it initiates an immune response via upregulation of microgliosis and recruits lymphocytes into the SN, prior to degeneration [for review (129)].

Autoimmunity arises from the failure to recognize endogenous proteins as self, driving the immune system to respond to and attack ones own cells and tissues. PD might result from an autoimmune response by recognizing aggregated, misprocessed α-syn as a foreign entity. α-Syn is both hyperphosphorylated and proteolytically misprocessed in the brains of PD patients, leading to its aggregation and deposition (130). Modified forms of α-syn are resistant to normal degradation by the proteasome and chaperone-mediate autophagy (CMA) (131). Proteolytic misprocessing of mutant or aggregated protein or post-translation modifications of α-syn in PD (131) could potentiate the formation of neo-epitopes.

Peptides derived from α-syn elicit *in vitro* responses from cytotoxic and Th cells from PD patients but not healthy controls (132). In addition, one of these peptide regions strongly binds to MHC complexes encoded by HLA (DRB1*15:01, DRB5*01:01) that are associated with PD by GWAS (105–109). The second region of these peptides requires phosphorlyaton at an α-syn residue, S129, that is found in high levels in Lewy bodies (133).

We note that these α-syn-specific T cells have thus far only been detected in the periphery of PD patients. If they were to extravasate into the brain, they would detect α-syn on the surface of microglia and neurons. Antigen recognition by Th1 and cytotoxic T cells leads to increased local inflammation and cell death. However, whether α-syn–specific T cells identified in PD patients permeate into the CNS and cause dopaminergic neuron death remains to be determined.

Implications for Diagnosis and Therapeutics

Understanding the extent of the role of the immune response in the pathogenesis of PD opens up new avenues of diagnosis and treatment for patients. Identifying people who are carriers of HLA alleles that place them at risk for α-syn-specific T cells to be monitored can help early diagnosis and treatment of PD. In addition, α-syn-specific T cells could be use as early biomarkers of the disease, identifying autoimmunity to self proteins prior to the onset of motor symptoms. Large-scale, longitudinal studies monitoring α-syn reactivity and the development of PD would need to be conducted before establishing T cells as PD biomarkers.

Currently, new treatment avenues targeting the immune system are being tested on PD patients. Sargramostim is a human recombinant granulocyte-macrophage colony-stimulating factor approved by the Food and Drug Administration for the recovery of patients receiving bone marrow transplantation and cancer therapies (134). It functions by promoting myeloid recovery and inducing Treg responses (134). In a preliminary randomized, double-blind phase 1 clinical trial, Sargramostim-treated patients showed modest improvements after 6 and 8 weeks of treatment and increases in Treg numbers and function compared to PD patients receiving placebo (135). Further clinical investigation will shed light on the potential of immunomodulatory drugs for treatment of PD.

Another approach to treatment currently being investigated is the targeting of α-syn with antibodies to slow the spread and reverse the effects of α-syn pathology. Monoclonal antibodies against α-syn have shown to reduce the levels of protein spread, ameliorate dopaminergic neuron loss, and attenuate motor deficits in PD mouse models (136, 137). Affitope PD01A and PRX002/RG7935 currently in clinical trial have shown to penetrate the BBB and lower plasma levels of α-syn (138, 139). However, it should be noted that anti-α-syn antibodies are shown to be present in the blood and cerebral spinal fluid of PD patients and absent in healthy controls (140–142). Therefore, anti-α-syn antibodies may be more promising as an early biomarker for PD rather than a treatment.

The immune system is increasingly acknowledged to play a larger role in propagating PD than previously thought and is a promising pathway for treatment. When designing immune-modulatory drugs, timing is important. If peripheral inflammation, α-syn aggregation, BBB leakage, and α-syn-specific T cells drive neuronal death, it is critical to treat PD patients before the activation of the autoimmune pathway. Immunomodulatory treatment would be most efficacious at

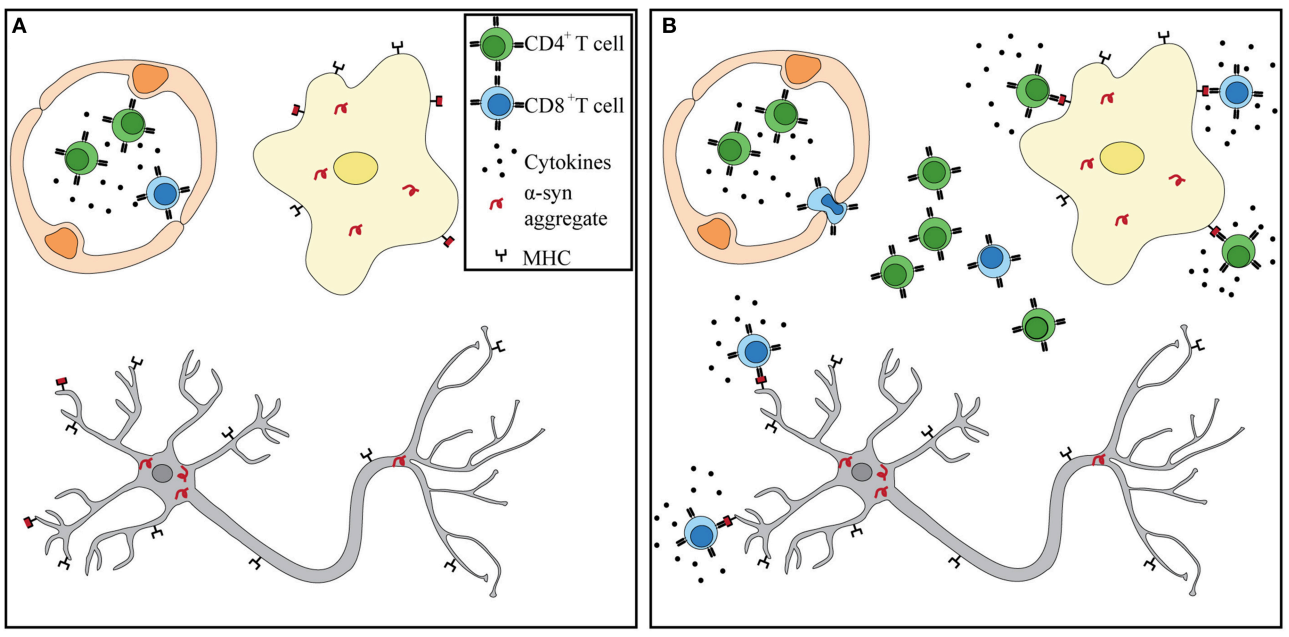

FIGURE 1 | α-Syn T cells infiltrate into the CNS and recognize antigen presented by microglia and dopaminergic neurons. **(A)** Prior to BBB damage, peripherally primed α-syn T cells circulate throughout the blood in the presence of proinflammatory cytokines. α-Syn aggregates have already formed in dopaminergic neurons and microglia. **(B)** Increased BBB permeability through either weakened tight junctions or increased transcytosis allows for extravasation of α-syn T cells. Recognition of α-syn presented by MHC-I on neurons and MHC-I and –II on microglia leads to T cell activation and release of granzymes and proinflammatory cytokines. Dopaminergic neurons eventually die in the presence of sustained inflammation and cytotoxic environment.

the onset of loss of α-syn tolerance, before immune cells have targeted dopaminergic cells. Developing biomarker tests to examine patients and identify α-syn-specific T cells early would provide a longer window for immunomodulatory treatments to stop PD progression in its tracks.

CONCLUSIONS AND FUTURE DIRECTIONS

Numerous independent studies suggest that a heightened and sustained immune response observed in PD patients may be a driver, rather than a consequence, of neuronal death. α-Syn pathology may begin in the periphery, where the enteric nervous system is compromised up to 20 years prior to diagnosis. Circulating T cells of PD patients have been shown to respond to α-syn stimulation *in vitro*. PD patients are afflicted with sustained peripheral inflammation, which has been shown to lead to a deterioration of the BBB. Lymphocytes can extravasate into the CNS via the BBB that is shown to be leaky in PD patients. Within the CNS, α-syn accumulation and spread, microgliosis, and antigen presentation can help recruit and attract α-syn -specific T cells. α-Syn -targeting $CD4^+$ and $CD8^+$ T cells observed in PD patients can recognize specific peptides bound to MHC-II on microglia and MHC-I on dopaminergic neurons (**Figure 1**). Further studies are necessary to test this hypothesis. However, that each phenomenon has been observed in PD patients and proven essential in recapitulating the disease in animal models lends support to its potential. Future studies should integrate α-syn-specificity and HLA association observed in PD patients with iPSCs neurons and antigen presenting cells to fully recapitulate what occurs in the CNS during disease. If α-syn-specific T cells are driving neurodegeneration in PD, then biomarkers, diagnostic tools, and treatments can be adapted to identifying autoimmune cells in patients and targeting them.

AUTHOR CONTRIBUTIONS

FG wrote the review. DA, CL, AS, and DS edited the review and provided feedback.

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REFERENCES

1. Amor S, Peferoen LA, Vogel DY, Breur M, van der Valk P, Baker D, et al. Inflammation in neurodegenerative diseases–an update. *Immunology* (2014) 142:151–66. doi: 10.1111/imm.12233
2. Dorsey ER, Constantinescu R, Thompson JP, Biglan KM, Holloway RG, Kieburtz K, et al. Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology* (2007) 68:384–6. doi: 10.1212/01.wnl.0000247740.47667.03
3. Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord*. (2015) 30:1591–601. doi: 10.1002/mds.26424
4. Billingsley KJ, Bandres-Ciga S, Saez-Atienzar S, Singleton AB. Genetic risk factors in Parkinson's disease. *Cell Tissue Res*. (2018) 373:9–20. doi: 10.1007/s00441-018-2817-y
5. Kalia LV, Lang AE. Parkinson's disease. *Lancet* (2015) 386:896–912. doi: 10.1016/S0140-6736(14)61393-3
6. Postuma RB, Aarsland D, Barone P, Burn DJ, Hawkes CH, Oertel W, et al. Identifying prodromal Parkinson's disease: pre-motor disorders in Parkinson's disease. *Mov Disord*. (2012) 27:617–26. doi: 10.1002/mds.24996
7. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, et al. Parkinson disease. *Nat Rev Dis Primers* (2017) 3:17013. doi: 10.1038/nrdp.2017.13
8. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* (2003) 24:197–211. doi: 10.1016/S0197-4580(02)00065-9
9. Abbott RD, Petrovitch H, White LR, Masaki KH, Tanner CM, Curb JD, et al. Frequency of bowel movements and the future risk of Parkinson's disease. *Neurology* (2001) 57:456–62. doi: 10.1212/WNL.57.3.456
10. Pont-Sunyer C, Hotter A, Gaig C, Seppi K, Compta Y, Katzenschlager R, et al. The onset of nonmotor symptoms in Parkinson's disease (the ONSET PD study). *Mov Disord*. (2015) 30:229–37. doi: 10.1002/mds.26077
11. Edwards LL, Pfeiffer RF, Quigley EM, Hofman R, Balluff M. Gastrointestinal symptoms in Parkinson's disease. *Mov Disord*. (1991) 6:151–6. doi: 10.1002/mds.870060211
12. Keshavarzian, Green SJ, Engen PA, Voigt RM, Naqib A, Forsyth CB, et al. Colonic bacterial composition in Parkinson's disease. *Mov Disord*. (2015) 30:1351–60. doi: 10.1002/mds.26307
13. Unger MM, Spiegel J, Dillmann KU, Grundmann D, Philippeit H, Burmann J, et al. Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. *Parkinsonism Relat Disord*. (2016) 32:66–72. doi: 10.1016/j.parkreldis.2016.08.019
14. Hasegawa S, Goto S, Tsuji H, Okuno T, Asahara T, Nomoto K, et al. Intestinal dysbiosis and lowered serum lipopolysaccharide-binding protein in Parkinson's disease. *PLoS ONE* (2015) 10:e0142164. doi: 10.1371/journal.pone.0142164
15. Scheperjans F, Aho V, Pereira PA, Koskinen K, Paulin L, Pekkonen E, et al. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov Disord*. (2015) 30:350–8. doi: 10.1002/mds.26069
16. Davies KN, King D, Billington D, Barrett JA. Intestinal permeability and orocaecal transit time in elderly patients with Parkinson's disease. *Postgraduate Med J*. (1996) 72:164–7. doi: 10.1136/pgmj.72.845.164
17. Salat-Foix D, Tran K, Ranawaya R, Meddings J, Suchowersky O. Increased intestinal permeability and Parkinson disease patients: chicken or egg? *Can J Neurol Sci*. (2012) 39:185–8. doi: 10.1017/S0317167100013202
18. Forsyth CB, Shannon KM, Kordower JH, Voigt RM, Shaikh M, Jaglin JA, et al. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PLoS ONE* (2011) 6:e28032. doi: 10.1371/journal.pone.0028032
19. Chen SG, Stribinskis V, Rane MJ, Demuth DR, Gozal E, Roberts AM, et al. Exposure to the functional bacterial amyloid protein curli enhances alpha-synuclein aggregation in aged fischer 344 rats and *Caenorhabditis elegans*. *Sci Rep*. (2016) 6:34477. doi: 10.1038/srep34477
20. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation

in a model of Parkinson's disease. *Cell* (2016) 167:1469–80 e12. doi: 10.1016/j.cell.2016.11.018
21. Braak H, Rub U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *J Neural Trans*. (2003) 110:517–36. doi: 10.1007/s00702-002-0808-2
22. Braak H, de Vos RA, Bohl J, Del Tredici K. Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. *Neurosci Lett*. (2006) 396:67–72. doi: 10.1016/j.neulet.2005.11.012
23. Shannon KM, Keshavarzian A, Mutlu E, Dodiya HB, Daian D, Jaglin JA, et al. Alpha-synuclein in colonic submucosa in early untreated Parkinson's disease. *Mov Disord*. (2012) 27:709–15. doi: 10.1002/mds.23838
24. Beach TG, Adler CH, Sue LI, Vedders L, Lue L, White Iii CL, et al. Arizona Parkinson's disease, multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. *Acta Neuropathol*. (2010) 119:689–702. doi: 10.1007/s00401-010-0664-3
25. Adler CH, Beach TG. Neuropathological basis of nonmotor manifestations of Parkinson's disease. *Mov Disord*. (2016) 31:1114–9. doi: 10.1002/mds.26605
26. Heikkila RE, Nicklas WJ, Vyas I, Duvoisin RC. Dopaminergic toxicity of rotenone and the 1-methyl-4-phenylpyridinium ion after their stereotaxic administration to rats: implication for the mechanism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity. *Neurosci Lett*. (1985) 62:389–94. doi: 10.1016/0304-3940(85)90580-4
27. Spivey A. Rotenone and paraquat linked to Parkinson's disease: human exposure study supports years of animal studies. *Environ Health Perspect*. (2011) 119:A259. doi: 10.1289/ehp.119-a259a
28. Pan-Montojo F, Anichtchik O, Dening Y, Knels L, Pursche S, Jung R, et al. Progression of Parkinson's disease pathology is reproduced by intragastric administration of rotenone in mice. *PloS ONE* (2010) 5:e8762. doi: 10.1371/journal.pone.0008762
29. Pan-Montojo F, Schwarz M, Winkler C, Arnhold M, O'Sullivan GA, Pal A, et al. Environmental toxins trigger PD-like progression via increased alpha-synuclein release from enteric neurons in mice. *Sci Rep*. (2012) 2:898. doi: 10.1038/srep00898
30. Devos D, Lebouvier T, Lardeux B, Biraud M, Rouaud T, Pouclet H, et al. Colonic inflammation in Parkinson's disease. *Neurobiol Dis*. (2013) 50:42–8. doi: 10.1016/j.nbd.2012.09.007
31. Chen Y, Yu M, Liu X, Qu H, Chen Q, Qian W, et al. Clinical characteristics and peripheral T cell subsets in Parkinson's disease patients with constipation. *Int J Clin Exp Pathol*. (2015) 8:2495–504.
32. Mogi M, Harada M, Narabayashi H, Inagaki H, Minami M, Nagatsu T. Interleukin (IL)-1 beta, IL-2, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. *Neurosci Lett*. (1996) 211:13–6. doi: 10.1016/0304-3940(96)12706-3
33. Stypula G, Kunert-Radek J, Stepien H, Zylinska K, Pawlikowski M. Evaluation of interleukins, ACTH, cortisol and prolactin concentrations in the blood of patients with parkinson's disease. *Neuroimmunomodulation* (1996) 3:131–4. doi: 10.1159/000097237
34. Blum-Degen D, Muller T, Kuhn W, Gerlach M, Przuntek H, Riederer P. Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and *de novo* Parkinson's disease patients. *Neurosci Lett*. (1995) 202:17–20. doi: 10.1016/0304-3940(95)12192-7
35. Dobbs RJ, Charlett A, Purkiss AG, Dobbs SM, Weller C, Peterson DW. Association of circulating TNF-alpha and IL-6 with ageing and parkinsonism. *Acta Neurol Scand*. (1999) 100:34–41. doi: 10.1111/j.1600-0404.1999.tb00721.x
36. Mogi M, Harada M, Kondo T, Riederer P, Inagaki H, Minami M, et al. Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from parkinsonian patients. *Neurosci Lett*. (1994) 180:147–50. doi: 10.1016/0304-3940(94)90508-8
37. Muller T, Blum-Degen D, Przuntek H, Kuhn W. Interleukin-6 levels in cerebrospinal fluid inversely correlate to severity of Parkinson's disease. *Acta Neurol Scand*. (1998) 98:142–4. doi: 10.1111/j.1600-0404.1998.tb01736.x
38. Reale M, Iarlori C, Thomas A, Gambi D, Perfetti B, Di Nicola M, et al. Peripheral cytokines profile in Parkinson's disease. *Brain Behav Immun*. (2009) 23:55–63. doi: 10.1016/j.bbi.2008.07.003
39. Rentzos M, Nikolaou C, Andreadou E, Paraskevas GP, Rombos A, Zoga M, et al. Circulating interleukin-15 and RANTES chemokine in Parkinson's disease. *Acta Neurol Scand*. (2007) 116:374–9. doi: 10.1111/j.1600-0404.2007.00894.x
40. Boka G, Anglade P, Wallach D, Javoy-Agid F, Agid Y, Hirsch EC. Immunocytochemical analysis of tumor necrosis factor and its receptors in Parkinson's disease. *Neurosci Lett*. (1994) 172:151–4. doi: 10.1016/0304-3940(94)90684-X
41. Hunot S, Boissiere F, Faucheux B, Brugg B, Mouatt-Prigent A, Agid Y, et al. Nitric oxide synthase and neuronal vulnerability in Parkinson's disease. *Neuroscience* (1996) 72:355–63. doi: 10.1016/0306-4522(95)00578-1
42. Kustrimovic N, Comi C, Magistrelli L, Rasini E, Legnaro M, Bombelli R, et al. Parkinson's disease patients have a complex phenotypic and functional Th1 bias: cross-sectional studies of CD4+ Th1/Th2/T17 and Treg in drug-naive and drug-treated patients. *J Neuroinflamm*. (2018) 15:205. doi: 10.1186/s12974-018-1248-8
43. Sommer, Maxreiter F, Krach F, Fadler T, Grosch J, Maroni M, et al. Th17 lymphocytes induce neuronal cell death in a human iPSC-based model of Parkinson's disease. *Cell Stem Cell* (2018) 23:123–31 e6. doi: 10.1016/j.stem.2018.06.015
44. Bas J, Calopa M, Mestre M, Mollevi DG, Cutillas B, Ambrosio S, et al. Lymphocyte populations in Parkinson's disease and in rat models of parkinsonism. *J Neuroimmunol*. (2001) 113:146–52. doi: 10.1016/S0165-5728(00)00422-7
45. Saunders JA, Estes KA, Kosloski LM, Allen HE, Dempsey KM, Torres-Russotto DR, et al. CD4+ regulatory and effector/memory T cell subsets profile motor dysfunction in Parkinson's disease. *J Neuroimmune Pharmacol*. (2012) 7:927–38. doi: 10.1007/s11481-012-9402-z
46. Calopa M, Bas J, Callen A, Mestre M. Apoptosis of peripheral blood lymphocytes in Parkinson patients. *Neurobiol Dis*. (2010) 38:1–7. doi: 10.1016/j.nbd.2009.12.017
47. Stevens CH, Rowe D, Morel-Kopp MC, Orr C, Russell T, Ranola M, et al. Reduced T helper and B lymphocytes in Parkinson's disease. *J Neuroimmunol*. (2012) 252:95–9. doi: 10.1016/j.jneuroim.2012.07.015
48. Chiba S, Matsumoto H, Saitoh M, Kasahara M, Matsuya M, Kashiwagi M. A correlation study between serum adenosine deaminase activities and peripheral lymphocyte subsets in Parkinson's disease. *J Neurol Sci*. (1995) 132:170–3. doi: 10.1016/0022-510X(95)00136-P
49. Baba Y, Kuroiwa A, Uitti RJ, Wszolek ZK, Yamada T. Alterations of T-lymphocyte populations in Parkinson disease. *Parkinsonism Relat Disord*. (2005) 11:493–8. doi: 10.1016/j.parkreldis.2005.07.005
50. Gruden MA, Sewell RD, Yanamandra K, Davidova TV, Kucheryanu VG, Bocharov EV, et al. Immunoprotection against toxic biomarkers is retained during Parkinson's disease progression. *J Neuroimmunol*. (2011) 233:221–7. doi: 10.1016/j.jneuroim.2010.12.001
51. Chen Y, Qi B, Xu W, Ma B, Li L, Chen Q, et al. Clinical correlation of peripheral CD4+cell subsets, their imbalance and Parkinson's disease. *Mol Med Rep*. (2015) 12:6105–11. doi: 10.3892/mmr.2015.4136
52. Rosenkranz D, Weyer S, Tolosa E, Gaenslen A, Berg D, Leyhe T, et al. Higher frequency of regulatory T cells in the elderly and increased suppressive activity in neurodegeneration. *J Neuroimmunol*. (2007) 188:117–27. doi: 10.1016/j.jneuroim.2007.05.011
53. Chen S, Liu Y, Niu Y, Xu Y, Zhou Q, Xu X, et al. Increased abundance of myeloid-derived suppressor cells and Th17 cells in peripheral blood of newly-diagnosed Parkinson's disease patients. *Neurosci Lett*. (2017) 648:21–5. doi: 10.1016/j.neulet.2017.03.045
54. Kondelkova K, Vokurkova D, Krejsek J, Borska L, Fiala Z, Ctirad A. Regulatory T cells (TREG) and their roles in immune system with respect to immunopathological disorders. *Acta Med*. (2010) 53:73–7. doi: 10.14712/18059694.2016.63
55. Liebner S, Dijkhuizen RM, Reiss Y, Plate KH, Agalliu D, Constantin G. Functional morphology of the blood-brain barrier in health and disease. *Acta Neuropathol*. (2018) 135:311–36. doi: 10.1007/s00401-018-1815-1
56. Daneman R, Prat A. The blood-brain barrier. *Cold Spring Harb Perspect Biol*. (2015) 7:a020412. doi: 10.1101/cshperspect.a020412

57. Varatharaj, Galea I. The blood-brain barrier in systemic inflammation. *Brain Behav. Immun.* (2017) 60:1–12. doi: 10.1016/j.bbi.2016.03.010
58. Daneman R, Engelhardt B. Brain barriers in health and disease. *Neurobiol Dis.* (2017) 107:1–3. doi: 10.1016/j.nbd.2017.05.008
59. Kortekaas R, Leenders KL, van Oostrom JC, Vaalburg W, Bart J, Willemsen AT, et al. Blood-brain barrier dysfunction in parkinsonian midbrain *in vivo*. *Ann Neurol.* (2005) 57:176–9. doi: 10.1002/ana.20369
60. Ham JH, Yi H, Sunwoo MK, Hong JY, Sohn YH, Lee PH. Cerebral microbleeds in patients with Parkinson's disease. *J Neurol.* (2014) 261:1628–35. doi: 10.1007/s00415-014-7403-y
61. Janelidze S, Lindqvist D, Francardo V, Hall S, Zetterberg H, Blennow K, et al. Increased CSF biomarkers of angiogenesis in Parkinson disease. *Neurology* (2015) 85:1834–42. doi: 10.1212/WNL.0000000000002151
62. Gray MT, Woulfe JM. Striatal blood-brain barrier permeability in Parkinson's disease. *J Cereb Blood Flow Metab.* (2015) 35:747–50. doi: 10.1038/jcbfm.2015.32
63. Pienaar IS, Lee CH, Elson JL, McGuinness L, Gentleman SM, Kalaria RN, et al. Deep-brain stimulation associates with improved microvascular integrity in the subthalamic nucleus in Parkinson's disease. *Neurobiol Dis.* (2015) 74:392–405. doi: 10.1016/j.nbd.2014.12.006
64. Desai Bradaric B, Patel A, Schneider JA, Carvey PM, Hendey B. Evidence for angiogenesis in Parkinson's disease, incidental Lewy body disease, and progressive supranuclear palsy. *J Neural Trans.* (2012) 119:59–71. doi: 10.1007/s00702-011-0684-8
65. McGeer PL, Itagaki S, Akiyama H, McGeer EG. Rate of cell death in parkinsonism indicates active neuropathological process. *Ann Neurol.* (1988) 24:574–6. doi: 10.1002/ana.410240415
66. McGeer PL, Itagaki S, McGeer EG. Expression of the histocompatibility glycoprotein HLA-DR in neurological disease. *Acta Neuropathol.* (1988) 76:550–7. doi: 10.1007/BF00689592
67. Brochard V, Combadiere B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, et al. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Investig.* (2009) 119:182–92. doi: 10.1172/JCI36470
68. Ungerstedt U. 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. *Eur J Pharmacol.* (1968) 5:107–10. doi: 10.1016/0014-2999(68)90164-7
69. Saner, Thoenen H. Model experiments on the molecular mechanism of action of 6-hydroxydopamine. *Mol Pharmacol.* (1971) 7:147–54.
70. Graham DG. Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. *Mol Pharmacol.* (1978) 14:633–43.
71. Blum D, Torch S, Lambeng N, Nissou M, Benabid AL, Sadoul R, et al. Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. *Prog Neurobiol.* (2001) 65:135–72. doi: 10.1016/S0301-0082(01)00003-X
72. Theodore S, Maragos W. 6-Hydroxydopamine as a tool to understand adaptive immune system-induced dopamine neurodegeneration in Parkinson's disease. *Immunopharmacol Immunotoxicol.* (2015) 37:393–9. doi: 10.3109/08923973.2015.1070172
73. Ambrosi G, Kustrimovic N, Siani F, Rasini E, Cerri S, Ghezzi C, et al. Complex changes in the innate and adaptive immunity accompany progressive degeneration of the nigrostriatal pathway induced by intrastriatal injection of 6-hydroxydopamine in the rat. *Neurotoxicity Res.* (2017) 32:71–81. doi: 10.1007/s12640-017-9712-2
74. Davis GC, Williams AC, Markey SP, Ebert MH, Caine ED, Reichert CM, et al. Chronic Parkinsonism secondary to intravenous injection of meperidine analogues. *Psychiatry Res.* (1979) 1:249–54. doi: 10.1016/0165-1781(79)90006-4
75. Langston JW, Ballard PA Jr. Parkinson's disease in a chemist working with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *N Eng J Med.* (1983) 309:310.
76. Cui M, Aras R, Christian WV, Rappold PM, Hatwar M, Panza J, et al. The organic cation transporter-3 is a pivotal modulator of neurodegeneration in the nigrostriatal dopaminergic pathway. *Proc Natl Acad Sci USA.* (2009) 106:8043–8. doi: 10.1073/pnas.0900358106
77. Rappold PM, Tieu K. Astrocytes and therapeutics for Parkinson's disease. *Neurotherapeutics* (2010) 7:413–23. doi: 10.1016/j.nurt.2010.07.001
78. Nicklas WJ, Vyas I, Heikkila RE. Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenyl-pyridine, a metabolite of the neurotoxin, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *Life Sci.* (1985) 36:2503–8. doi: 10.1016/0024-3205(85)90146-8
79. Mizuno Y, Sone N, Saitoh T. Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 1-methyl-4-phenylpyridinium ion on activities of the enzymes in the electron transport system in mouse brain. *J Neurochem.* (1987) 48:1787–93. doi: 10.1111/j.1471-4159.1987.tb05737.x
80. Choi SJ, Panhelainen A, Schmitz Y, Larsen KE, Kanter E, Wu M, et al. Changes in neuronal dopamine homeostasis following 1-methyl-4-phenylpyridinium (MPP+) exposure. *J Biol Chem.* (2015) 290:6799–809. doi: 10.1074/jbc.M114.631556
81. Lieberman OJ, Choi SJ, Kanter E, Saverchenko A, Frier MD, Fiore GM, et al. alpha-synuclein-dependent calcium entry underlies differential sensitivity of cultured SN and VTA dopaminergic neurons to a Parkinsonian neurotoxin. *eNeuro* (2017) 4:ENEURO.0167-17.2017. doi: 10.1523/ENEURO.0167-17.2017
82. Kurkowska-Jastrzebska, Wronska A, Kohutnicka M, Czlonkowski A, Czlonkowska A. MHC class II positive microglia and lymphocytic infiltration are present in the substantia nigra and striatum in mouse model of Parkinson's disease. *Acta Neurobiol Exp.* (1999) 59:1–8.
83. Depboylu C, Stricker S, Ghobril JP, Oertel WH, Priller J, Hoglinger GU. Brain-resident microglia predominate over infiltrating myeloid cells in activation, phagocytosis and interaction with T-lymphocytes in the MPTP mouse model of Parkinson disease. *Exp. Neurol.* (2012) 238:183–91. doi: 10.1016/j.expneurol.2012.08.020
84. Benner EJ, Banerjee R, Reynolds AD, Sherman S, Pisarev VM, Tsiperson V, et al. Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. *PloS ONE* (2008) 3:e1376. doi: 10.1371/journal.pone.0001376
85. Nagai Y, Ueno S, Saeki Y, Soga F, Hirano M, Yanagihara T. Decrease of the D3 dopamine receptor mRNA expression in lymphocytes from patients with Parkinson's disease. *Neurology* (1996) 46:791–5. doi: 10.1212/WNL.46.3.791
86. Gonzalez H, Contreras F, Prado C, Elgueta D, Franz D, Bernales S, et al. Dopamine receptor D3 expressed on CD4+ T cells favors neurodegeneration of dopaminergic neurons during Parkinson's disease. *J Immunol.* (2013) 190:5048–56. doi: 10.4049/jimmunol.1203121
87. Contreras F, Prado C, Gonzalez H, Franz D, Osorio-Barrios F, Osorio F, et al. Dopamine receptor D3 signaling on CD4+ T cells favors Th1- and Th17-mediated immunity. *J Immunol.* (2016) 196:4143–9. doi: 10.4049/jimmunol.1502420
88. Benner EJ, Mosley RL, Destache CJ, Lewis TB, Jackson-Lewis V, Gorantla S, et al. Therapeutic immunization protects dopaminergic neurons in a mouse model of Parkinson's disease. *Proc Natl Acad Sci USA.* (2004) 101:9435–40. doi: 10.1073/pnas.0400569101
89. Reynolds AD, Banerjee R, Liu J, Gendelman HE, Mosley RL. Neuroprotective activities of CD4+CD25+ regulatory T cells in an animal model of Parkinson's disease. *J Leukoc Biol.* (2007) 82:1083–94. doi: 10.1189/jlb.0507296
90. Reynolds AD, Stone DK, Mosley RL, Gendelman HE. Nitrated {alpha}-synuclein-induced alterations in microglial immunity are regulated by CD4+ T cell subsets. *J Immunol.* (2009) 182:4137–49. doi: 10.4049/jimmunol.0803982
91. Reynolds AD, Stone DK, Hutter JA, Benner EJ, Mosley RL, Gendelman HE. Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. *J Immunol.* (2010) 184:2261–71. doi: 10.4049/jimmunol.0901852
92. Theodore S, Cao S, McLean PJ, Standaert DG. Targeted overexpression of human alpha-synuclein triggers microglial activation and an adaptive immune response in a mouse model of Parkinson disease. *J Neuropathol Exp Neurol.* (2008) 67:1149–58. doi: 10.1097/NEN.0b013e31818e5e99
93. Van der Perren, Macchi F, Toelen J, Carlon MS, Maris M, de Loor H, et al. FK506 reduces neuroinflammation and dopaminergic neurodegeneration in an alpha-synuclein-based rat model for Parkinson's disease. *Neurobiol Aging* (2015) 36:1559–68. doi: 10.1016/j.neurobiolaging.2015.01.014
94. Reich DS, Lucchinetti CF, Calabresi PA. Multiple sclerosis. *N Eng J Med.* (2018) 378:169–80. doi: 10.1056/NEJMra1401483

95. Hemmer B, Kerschensteiner M, Korn T. Role of the innate and adaptive immune responses in the course of multiple sclerosis. *Lancet Neurol*. (2015) 14:406–19. doi: 10.1016/S1474-4422(14)70305-9
96. Li YF, Zhang SX, Ma XW, Xue YL, Gao C, Li XY. Levels of peripheral Th17 cells and serum Th17-related cytokines in patients with multiple sclerosis: a meta-analysis. *Multiple Sclerosis Relat Disord*. (2017) 18:20–5. doi: 10.1016/j.msard.2017.09.003
97. Siffrin V, Radbruch H, Glumm R, Niesner R, Paterka M, Herz J, et al. *In vivo* imaging of partially reversible th17 cell-induced neuronal dysfunction in the course of encephalomyelitis. *Immunity* (2010) 33:424–36. doi: 10.1016/j.immuni.2010.08.018
98. Kang Z, Wang C, Zepp J, Wu L, Sun K, Zhao J, et al. Act1 mediates IL-17-induced EAE pathogenesis selectively in NG2+ glial cells. *Nat Neurosci*. (2013) 16:1401–8. doi: 10.1038/nn.3505
99. Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, et al. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat Med*. (2007) 13:1173–5. doi: 10.1038/nm1651
100. Codarri L, Gyulveszi G, Tosevski V, Hesske L, Fontana A, Magnenat L, et al. RORgammat drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. *Nat Immunol*. (2011) 12:560–7. doi: 10.1038/ni.2027
101. El-Behi M, Ciric B, Dai H, Yan Y, Cullimore M, Safavi F, et al. The encephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF. *Nat Immunol*. (2011) 12:568–75. doi: 10.1038/ni.2031
102. Croxford AL, Lanzinger M, Hartmann FJ, Schreiner B, Mair F, Pelczar P, et al. The cytokine GM-CSF drives the inflammatory signature of CCR2+ monocytes and licenses autoimmunity. *Immunity* (2015) 43:502–14. doi: 10.1016/j.immuni.2015.08.010
103. Ajami B, Bennett JL, Krieger C, McNagny KM, Rossi FM. Infiltrating monocytes trigger EAE progression, but do not contribute to the resident microglia pool. *Nat Neurosci*. (2011) 14:1142–9. doi: 10.1038/nn.2887
104. Cebrian C, Zucca FA, Mauri P, Steinbeck JA, Studer L, Scherzer CR, et al. MHC-I expression renders catecholaminergic neurons susceptible to T-cell-mediated degeneration. *Nat Commun*. (2014) 5:3633. doi: 10.1038/ncomms4633
105. Wissemann WT, Hill-Burns EM, Zabetian CP, Factor SA, Patsopoulos N, Hoglund B, et al. Association of Parkinson disease with structural and regulatory variants in the HLA region. *Am J Hum Genet*. (2013) 93:984–93. doi: 10.1016/j.ajhg.2013.10.009
106. Hamza TH, Zabetian CP, Tenesa A, Laederach A, Montimurro J, Yearout D, et al. Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. *Nat Genet*. (2010) 42:781–5. doi: 10.1038/ng.642
107. Greenbaum J, Sidney J, Chung J, Brander C, Peters B, Sette A. Functional classification of class II human leukocyte antigen (HLA) molecules reveals seven different supertypes and a surprising degree of repertoire sharing across supertypes. *Immunogenetics* (2011) 63:325–35. doi: 10.1007/s00251-011-0513-0
108. Kannarkat GT, Cook DA, Lee JK, Chang J, Chung J, Sandy E, et al. Common genetic variant association with altered HLA expression, synergy with pyrethroid exposure, and risk for Parkinson's disease: an Observational and Case-Control Study. *NPJ Parkinson's Dis*. (2015) 1:15002. doi: 10.1038/npjparkd.2015.2
109. Hill-Burns EM, Wissemann WT, Hamza TH, Factor SA, Zabetian CP, Payami H. Identification of a novel Parkinson's disease locus via stratified genome-wide association study. *BMC Genomics* (2014) 15:118. doi: 10.1186/1471-2164-15-118
110. Imamura K, Hishikawa N, Sawada M, Nagatsu T, Yoshida M, Hashizume Y. Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. *Acta Neuropathol*. (2003) 106:518–26. doi: 10.1007/s00401-003-0766-2
111. Harms AS, Cao S, Rowse AL, Thome AD, Li X, Mangieri LR, et al. MHCII is required for alpha-synuclein-induced activation of microglia, CD4 T cell proliferation, and dopaminergic neurodegeneration. *J Neurosci*. (2013) 33:9592–600. doi: 10.1523/JNEUROSCI.5610-12.2013
112. Martin HL, Santoro M, Mustafa S, Riedel G, Forrester JV, Teismann P. Evidence for a role of adaptive immune response in the disease pathogenesis of the MPTP mouse model of Parkinson's disease. *Glia* (2016) 64:386–95. doi: 10.1002/glia.22935
113. Bryant CE, Orr S, Ferguson B, Symmons MF, Boyle JP, Monie TP. International Union of Basic and Clinical Pharmacology. XCVI. Pattern recognition receptors in health and disease. *Pharmacol Rev*. (2015) 67:462–504. doi: 10.1124/pr.114.009928
114. Hughes CD, Choi ML, Ryten M, Hopkins L, Drews A, Botia JA, et al. Correction to: Picomolar concentrations of oligomeric alpha-synuclein sensitizes TLR4 to play an initiating role in Parkinson's disease pathogenesis. *Acta Neuropathol*. (2018) 137:121. doi: 10.1007/s00401-018-1919-7
115. Zhang W, Phillips K, Wielgus AR, Liu J, Albertini A, Zucca FA, et al. Neuromelanin activates microglia and induces degeneration of dopaminergic neurons: implications for progression of Parkinson's disease. *Neurotoxic Res*. (2011) 19:63–72. doi: 10.1007/s12640-009-9140-z
116. Grozdanov V, Bliederhaeuser C, Ruf WP, Roth V, Fundel-Clemens K, Zondler L, et al. Inflammatory dysregulation of blood monocytes in Parkinson's disease patients. *Acta Neuropathol*. (2014) 128:651–63. doi: 10.1007/s00401-014-1345-4
117. Stolzenberg E, Berry D, Yang, Lee EY, Kroemer A, Kaufman S, et al. A role for neuronal alpha-synuclein in gastrointestinal immunity. *J Innate Immun*. (2017) 9:456–63. doi: 10.1159/000477990
118. Ransohoff RM. Microglia and monocytes: 'tis plain the twain meet in the brain. *Nat Neurosci*. (2011) 14:1098–100. doi: 10.1038/nn.2917
119. Harms AS, Thome AD, Yan Z, Schonhoff AM, Williams GP, Li X, et al. Peripheral monocyte entry is required for alpha-Synuclein induced inflammation and Neurodegeneration in a model of Parkinson disease. *Exp Neurol*. (2018) 300:179–87. doi: 10.1016/j.expneurol.2017.11.010
120. Shemer, Erny D, Jung S, Prinz M. Microglia plasticity during health and disease: an immunological perspective. *Trends Immunol*. (2015) 36:614–24. doi: 10.1016/j.it.2015.08.003
121. Gerhard, Pavese N, Hotton G, Turkheimer F, Es M, Hammers A, Eggert K, et al. *In vivo* imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiol. Dis*. (2006) 21:404–12. doi: 10.1016/j.nbd.2005.08.002
122. Ouchi Y, Yoshikawa E, Sekine Y, Futatsubashi M, Kanno T, Ogusu T, et al. Microglial activation and dopamine terminal loss in early Parkinson's disease. *Ann Neurol*. (2005) 57:168–75. doi: 10.1002/ana.20338
123. Roy A, Mondal S, Kordower JH, Pahan K. Attenuation of microglial RANTES by NEMO-binding domain peptide inhibits the infiltration of CD8(+) T cells in the nigra of hemiparkinsonian monkey. *Neuroscience* (2015) 302:36–46. doi: 10.1016/j.neuroscience.2015.03.011
124. Chandra G, Rangasamy SB, Roy A, Kordower JH, Pahan K. Neutralization of RANTES and eotaxin prevents the loss of dopaminergic neurons in a mouse model of parkinson disease. *J Biol Chem*. (2016) 291:15267–81. doi: 10.1074/jbc.M116.714824
125. Tristao FS, Lazzarini M, Martin S, Amar M, Stuhmer W, Kirchhoff F, et al. CX3CR1 disruption differentially influences dopaminergic neuron degeneration in Parkinsonian mice depending on the neurotoxin and route of administration. *Neurotoxic Res*. (2016) 29:364–80. doi: 10.1007/s12640-015-9557-5
126. Parillaud VR, Lornet G, Monnet Y, Privat AL, Haddad AT, Brochard V, et al. Analysis of monocyte infiltration in MPTP mice reveals that microglial CX3CR1 protects against neurotoxic over-induction of monocyte-attracting CCL2 by astrocytes. *J Neuroinflamm*. (2017) 14:60. doi: 10.1186/s12974-017-0830-9
127. Sadeghian M, Mullali G, Pocock JM, Piers T, Roach A, Smith KJ. Neuroprotection by safinamide in the 6-hydroxydopamine model of Parkinson's disease. *Neuropathol Appl Neurobiol*. (2016) 42:423–35. doi: 10.1111/nan.12263
128. Zhang S, Wang XJ, Tian LP, Pan J, Lu GQ, Zhang YJ, et al. CD200-CD200R dysfunction exacerbates microglial activation and dopaminergic neurodegeneration in a rat model of Parkinson's disease. *J Neuroinflamm*. (2011) 8:154. doi: 10.1186/1742-2094-8-154
129. Sanchez-Guajardo V, Barnum CJ, Tansey MG, Romero-Ramos M. Neuroimmunological processes in Parkinson's disease and their relation to alpha-synuclein: microglia as the referee between neuronal

processes and peripheral immunity. *ASN Neuro* (2013) 5:113–39. doi: 10.1042/AN20120066
130. Przuntek H, Muller T, Riederer P. Diagnostic staging of Parkinson's disease: conceptual aspects. *J Neural Trans*. (2004) 111:201–16. doi: 10.1007/s00702-003-0102-y
131. Cuervo AM, Wong ES, Martinez-Vicente M. Protein degradation, aggregation, and misfolding. *Mov Disord*. (2010) 25 (Suppl. 1) S49–54. doi: 10.1002/mds.22718
132. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, et al. T cells from patients with Parkinson's disease recognize alpha-synuclein peptides. *Nature* (2017) 546:656–61. doi: 10.1038/nature22815
133. Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M. alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. *Proc Natl Acad Sci USA*. (1998) 95:6469–73. doi: 10.1073/pnas.95.11.6469
134. Rowin J, Thiruppathi M, Arhebamen E, Sheng J, Prabhakar BS, Meriggioli MN. Granulocyte macrophage colony-stimulating factor treatment of a patient in myasthenic crisis: effects on regulatory T cells. *Muscle Nerve* (2012) 46:449–53. doi: 10.1002/mus.23488
135. Gendelman HE, Zhang Y, Santamaria P, Olson KE, Schutt CR, Bhatti D, et al. Evaluation of the safety and immunomodulatory effects of sargramostim in a randomized, double-blind phase 1 clinical Parkinson's disease trial. *NPJ Parkinson's Dis*. (2017) 3:10. doi: 10.1038/s41531-017-0013-5
136. Games D, Valera E, Spencer B, Rockenstein E, Mante M, Adame A, et al. Reducing C-terminal-truncated alpha-synuclein by immunotherapy attenuates neurodegeneration and propagation in Parkinson's disease-like models. *J Neurosci*. (2014) 34:9441–54. doi: 10.1523/JNEUROSCI.5314-13.2014
137. Lindstrom V, Fagerqvist T, Nordstrom E, Eriksson F, Lord A, Tucker S, et al. Immunotherapy targeting alpha-synuclein protofibrils reduced pathology in (Thy-1)-h[A30P] alpha-synuclein mice. *Neurobiol. Dis*. (2014) 69:134–43. doi: 10.1016/j.nbd.2014.05.009
138. Jankovic J, Goodman I, Safirstein B, Marmon TK, Schenk DB, Koller M, et al. Safety and tolerability of multiple ascending doses of PRX002/RG7935, an anti-alpha-synuclein monoclonal antibody, in patients with Parkinson disease: a randomized clinical trial. *JAMA Neurol*. (2018) 75:1206–14. doi: 10.1001/jamaneurol.2018.1487
139. Foltynie T, Langston JW. Therapies to slow, stop, or reverse Parkinson's disease. *J Parkinson's Dis*. (2018) 8:S115–21. doi: 10.3233/JPD-181481
140. Horvath, Iashchishyn IA, Forsgren L, Morozova-Roche LA. Immunochemical detection of alpha-synuclein autoantibodies in Parkinson's disease: correlation between plasma and cerebrospinal fluid levels. *ACS Chem Neurosci*. (2017) 8:1170–6. doi: 10.1021/acschemneuro.7b00063
141. Shalash A, Salama M, Makar M, Roushdy T, Elrassas HH, Mohamed W, et al. Elevated serum alpha-synuclein autoantibodies in patients with Parkinson's disease relative to Alzheimer's disease and controls. *Front Neurol*. (2017) 8:720. doi: 10.3389/fneur.2017.00720
142. Akhtar RS, Licata JP, Luk KC, Shaw LM, Trojanowski JQ, Lee VM. Measurements of auto-antibodies to alpha-synuclein in the serum and cerebral spinal fluids of patients with Parkinson's disease. *J Neurochem*. (2018) 145:489–503. doi: 10.1111/jnc.14330

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REVIEW
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Autoimmunity After Ischemic Stroke and Brain Injury

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Ischemic Stroke is a major cause of morbidity and mortality worldwide. Sterile inflammation occurs after both stroke subtypes and contributes to neuronal injury and damage to the blood-brain barrier with release of brain antigens and a potential induction of autoimmune responses that escape central and peripheral tolerance mechanisms. In stroke patients, the detection of T cells and antibodies specific to neuronal antigens suggests a role of humoral adaptive immunity. In experimental models stroke leads to a significant increase of autoreactive T and B cells to CNS antigens. Lesion volume and functional outcome in stroke patients and murine stroke models are connected to antigen-specific responses to brain proteins. In patients with traumatic brain injury (TBI) a range of antibodies against brain proteins can be detected in serum samples. In this review, we will summarize the role of autoimmunity in post-lesional conditions and discuss the role of B and T cells and their potential neuroprotective or detrimental effects.

Keywords: autoimmunity, traumatic brain injuries (TBI), autoantibodies, ischemic stroke (IS), adaptive immunity, neural antigens, autoreactive T and B cells, neuroinflammation

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INTRODUCTION

Ischemic Stroke and traumatic brain injury (TBI) cause damage to neurons, glia and the vasculature resulting in the disruption of the blood-brain barrier (BBB), hemorrhage, edema, and necrotic cell death. Cell injury and cell death lead to the release of danger signals that activate the immune system (1, 2). The subsequent sterile inflammatory reaction involves the innate immune system with activation of resident immune cells of the central nervous system (CNS) and a rapid infiltration of peripheral immune cells into the brain (3, 4). The presence of brain-derived antigens in the lymphoid tissue of stroke patients (5) and increased levels of brain antigens in the CNS and peripheral circulation in both diseases may trigger an adaptive autoimmune response. This process would require the presentation of antigen by antigen-presenting cells (APCs) to autoreactive T cells. The prevention of detrimental autoimmunity is usually mediated by central and peripheral tolerance mechanisms which include anergy, clonal deletion and suppression by regulatory T cells (Tregs) (6). Self-reactive T and/or B lymphocytes and/or their ability of autoantibody production can exist as physiological autoimmunity with no evidence of clinical disease, demonstrated by the presence of natural autoantibodies that are involved in homeostasis by degrading self- and foreign antigens (7). In the inflammatory microenvironment following stroke and TBI, self-reactive lymphocytes and autoantibodies could be generated and participate in inflammation, when immune tolerance is broken, leading to pathological autoimmunity, and further tissue injury. This antigen-dependent adaptive autoimmune response would therefore differ from the reactive innate immune response that involves antigen-independent deleterious effects of T cells found in acute experimental stroke (8). The presence of brain-reactive antibodies in stroke and TBI could be therefore an indication of an autoimmune response and multiple investigations now demonstrate their correlation to lesion size and functional outcome in stroke and TBI.

ANTIGEN SPECIFICITY IN ISCHEMIC STROKE

Ischemic stroke leads to a sterile inflammatory response with the accumulation of microglia, and infiltration of macrophages, lymphocytes, and dendritic cells (DCs) followed by neutrophils (3). T lymphocytes can be found in human post-mortem stroke tissue to a small extent in necrotic brain parenchyma and more consistently in perivascular spaces bordering acute infarcts (9). The activation of T lymphocytes via an antigen dependent or independent process is still controversial and the mechanisms of T cell activation after brain injury are not clearly known (10).

Stroke causes the disruption of the blood-brain barrier (BBB): Infiltrating neutrophils, major promoters of BBB breakdown, through the release of metalloproteinases (MMP) such as MMP-9, together with inflammatory mediators, lead to the degradation of tight junction (TJ) proteins (11, 12). This first pathway could allow for the release of brain specific antigens into the peripheral vasculature, depending on the degree of BBB permeability and the size of brain antigens. A second pathway through the glymphatic system could allow for cerebrospinal fluid (CSF) drainage of interstitial fluid (ISF), containing extracellular solutes such as brain antigens, from the brain parenchyma, along paravenous pathways into the lymphatic circulation, eventually reaching the lymph nodes (13, 14). The brain lymphatic vessels lining the dural sinuses, drain to the deep cervical lymph nodes with the capability of carrying fluids, immune cells, and possibly brain antigens from the CSF (15, 16).

The accumulation of APCs and the upregulation of MHC II molecules in the ischemic brain (1, 17, 18) coinciding with the peak infiltration of lymphocytes into the brain (3), could allow for an antigen-dependent T cell expansion in the brain itself. In experimental ischemic stroke, clonal T cell expansion could be detected after 7 and 14 days following stroke (19). APCs with brain antigens could also travel through the glymphatic route and brain meningeal lymphatic vessels into the lymph nodes. Providing that significantly more brain-derived antigens can be found in cervical lymph nodes of stroke patients (5), monkey experimental autoimmune encephalomyelitis (EAE) and individuals with multiple sclerosis (MS) (20) than in controls, the possibility of T cell reactivity to autoantigens through activation by antigen-presenting cells (APC) in the periphery and subsequent migration into the brain also exists. T cells monitoring the leptomeningeal surfaces could also be activated by CNS antigens presented by phagocytes, or re-stimulated by CNS antigens after being primed by antigens in the periphery (21, 22).

The development of an immune response or tolerance to brain antigens presented by APCs in lymph nodes would depend on several factors such as the activation status of APCs, costimulatory molecules, the environment of antigen presentation, or even a genetic predisposition to autoimmune disease (23, 24).

The role of dendritic cells (DCs), as professional antigen presenting cells, therefore deserves closer attention. DCs play a central role in maintaining immune tolerance. In a non-inflammatory environment, immature DCs present self-antigens to T cells, leading to anergy or deletion of T cells to avoid autoimmunity. Immature DCs have also been found in the meninges and the choroid plexus of the CNS sampling the CSF for antigens (25). The activation of DCs by pathogen or danger-associated molecular patterns (DAMPs), leads to DC maturation and migration into draining lymph or the ischemic brain, with an increase of MHC-II and co-stimulatory proteins, relevant for T cell activation (26, 27). In experimental autoimmune encephalomyelitis (EAE), different subtypes of activated DCs with upregulated levels of costimulatory molecules are abundantly present in inflamed CNS lesions and CSF, driving proinflammatory T cell responses (28, 29). The model of EAE is induced by the priming and expansion of CD4+ T cells responding to CNS myelin antigens and the disease is initiated by re-presentation of myelin epitopes by APCs (30). Peripherally derived myeloid dendritic cells (mDCs) in EAE were found to present endogenous myelin peptide, activating naive autoreactive pathogenic CD4+ T cells to proliferate and produce IL-17 (30). Recently, we showed that the ischemic brain is rapidly infiltrated by $IRF4^+/CD172a^+$ conventional type 2, IL-17 inducing DCs. These cells promote neutrophil infiltrating into ischemic hemispheres via induction of IL-17 in γδ T cells, which can be activated in a T cell receptor-independent manner (27). The question whether this DC population or another subtype present in the stroke brain, could mitigate an autoimmune response to brain antigens comparable to EAE, escaping tolerance mechanisms or becoming activated after re-encountering naturally occurring antibodies, therefore warrants attention in further studies.

Microglia, as the major resident immune cells in the brain become activated through proinflammatory signaling as a result of brain ischemia (31). Activated microglia are involved in cytokine production, phagocytosis, and antigen presentation (1). In multiple sclerosis (MS) patients and its animal models (EAE), activated microglia and infiltrating T cells are found in CNS lesion (32). Activated microglia act as APCs by expressing MHC class I and II together with costimulatory molecules (33). Their role as antigen-presenting cells in stroke has received only little attention until recently. In a study of experimental brain ischemia, microglia-like cells acted as the main antigen-presenting cells for myelin oligodendrocyte glycoprotein (MOG) antigens, leading to the expansion of previously transferred 2D2 reactive CD4+ T cells. Higher frequencies of MOG-specific T cells were found in the brain at day 4 and 7 after middle cerebral artery occlusion (MCAO) (34). MHC class II/TCR interaction was necessary for T cell expansion in the brain with the highest expression of MHC class II found on microglia-like cells. The T cell expansion was dependent on cerebral ischemia. This antigen-dependent T cell response resulted in increased neurodeficits and infarction volumes starting at day 4 after MCAO (34).

The time course of immune cell infiltration after stroke needs to be considered in distinguishing between possible antigen-dependent autoimmune and antigen-independent responses. Lymphocyte infiltration after experimental stroke has been suggested to take place as early as 3 h for CD8+ T cells and 24 h for CD4+ T cells (35–37), while other publications report

a delayed peak of CD4+ T cells at 3 days (3, 38) or 5 days (39). B and T lymphocytes are present in different models of stroke up to 7 weeks after stroke onset (40).

T cells in the acute phase of experimental stroke have been linked to lesion volume and functional outcome (1, 41). Transgenic mice deficient of lymphocytes and antibody-mediated depletion of CD4+, CD8+, and γt T cells have been associated with smaller infarcts and better functional outcome (8, 39, 42–45).

A possible antigen-specific T cell response to brain antigens however would take place several days after stroke according to the time that antigen uptake, processing, presentation and finally lymphocyte proliferation would need. The attenuation of secondary stroke injury after experimental stroke by interventions targeted at T cells has been repeatedly shown to be within the first days. Early detrimental lymphocyte invasion has been therefore deemed antigen-independent within the first days after stroke onset (8).

Fittingly, a study of tMCAo stroke in mice, showed a significant autoimmune response to brain antigens at 4 days through 10 days after stroke, determined by an increase in autoreactive CD4+ and CD8+ T cells and CD19+ B cells, isolated from spleens and cervical lymph nodes (46). The peak of autoreactivity was reported to be at 8 days in splenic CD4+ T cells and at day 10 for CD8+ T cells in the cervical lymph nodes. An increased autoreactive response to microtubule-associated protein 2 (MAP2) and myelin derived peptides such as myelin basic protein (MBP) and MOG was associated with smaller infarct volumes and an increased autoreactive response to the anti N-methyl-D-aspartate receptor 2A (anti-NMDAR2A) with larger infarct volumes in mice (46).

In contrast to (46), a study of brain-derived antigens in the cervical lymph nodes and palatine tonsil of 22 patients with acute stroke, found increased immunoreactivity to neuronal MAP2 and NMDA NR-2A to be associated with smaller infarct volumes at day 7 and better outcome, whereas increased immunoreactivity to myelin-derived MBP was associated with more severe impairments on admission, larger infarcts and worse outcome at clinical follow-up (5). Cells immunoreactive to neural antigens were mostly CD68+ macrophages expressing costimulatory MHC II receptors and their co-localization with CD69+ T cells suggested activation of T cells (5).

There is further evidence for detrimental antigen-dependent effects: CD8+ T cells with a ovalbumin-specific T cell receptor were transferred into Rag1 mice showing no activation, less brain infiltration and smaller infarct sizes at 7 days after pMCAO than after the adoptive transfer of wild-type CD8+ T cells which could function antigen-dependently (45).

Another important question is the role of regulatory T cells (Tregs). The role of Tregs in the regulation of lymphocytic activity especially in light of exposure to neural antigens has not been fully answered. Tregs have a role in controlling autoreactive responses and have been shown to inhibit autoimmunity, maintaining peripheral tolerance (47). Following stroke, an early infiltration of Tregs (CD25+Foxp3+) was observed within the first week after MCAO in mice, constituting 20% of all CD4+ T cells by Liesz et al. (48), while we found only <5% of all CD4+ T cells to be Tregs within the first week (3). Accordingly, (49) found a substantial infiltration of Tregs to be at 14 and 30 days as compared to 7 days after MCAO. Tregs, despite their delayed infiltration, have been nevertheless found to impact stroke outcomes within the first days after ischemia. It has been suggested that Tregs could target cells of the peripheral immune system early after ischemia, suppressing peripheral T cell activation or inhibiting autoantigen-specific clonal expansion (50). Accordingly, an increase of Tregs can be seen in the spleen following MCAO with possible implications on post-stroke immunosuppression (51). The question whether Tregs in stroke are neuroprotective or detrimental also remains disputed (52). A recent review found the majority of studies with Tregs targeted therapies to support the beneficial role of Tregs, with reduction of lesion size. Treg depletion models were more likely to show an increased infarct volume and increased leukocyte brain invasion and proinflammatory cytokine secretion, however some studies showed no effect on infarct volume (50). The difference in outcome has been attributed to the different intensity of neuroinflammation dependent on the lesion sizes in the use of different ischemia models (50). In a recent study, a substantial accumulation of Tregs in the mouse brain was detected starting a week after ischemic stroke, potentiating neurological recovery. Tregs transferred from ischemic mice accumulated more efficiently in the brain of lymphocyte deficient Rag2 $^{-/-}$ mice than Tregs from sham operated mice, suggesting the importance of antigen recognition for Treg cell expansion in the brain (53). The involvement of Tregs in suppressing autoimmune responses to neural antigens can be deduced from studies that use tolerization to brain antigens, showing an increased probability of Treg responses and improved neurological outcome (54–56). These studies are discussed further below.

Taken together, there evidence of an antigen-specific T cell presence in ischemic stroke. This develops within several days and decreases over time. It is unclear however if these findings are relevant to the human stroke.

T CELLS: ANTIGENS AND TOLERANCE IN STROKE

There is evidence for T cell reactivity to autoantigens such as myelin basic protein (MBP) and myelin proteolipid protein (PLP) in patients with ischemic stroke (57). Higher peak concentrations of MBP, neuron-specific enolase (NSE) and S100B in the serum of patients at 24 h after stroke have been associated with stroke severity and larger infarct volumes (58).

If T cells, particularly CD4+ T cells, encounter an antigen, they can react in several ways. Depending on the cytokine milieu the effector function of T cells will be shaped (59). For example, a TH1 type response, a TH2/TH3 response, a TH17 response, an activation-induced cell death or no immune response can follow. A TH1 response (sensitization) leads to the secretion of proinflammatory cytokines after reencountering with the sensitizing antigen. A TH2/TH3 response (tolerance) leads to the secretion of immunomodulatory cytokines after reencountering with the antigen. Stroke induces an early systemic

inflammatory response, which could promote sensitization to brain antigens in an altered microenvironment (60). It is believed that mucosal antigen administration shifts the immune response from a TH1 response with secretion of proinflammatory cytokines (e.g., IL-1b, TNF-α, IFN-γ) to a TH2 or TH3 response, with secretion of anti-inflammatory cytokines (e.g., TGF-b1, IL-10) that would limit cell-mediated responses and enhance humoral responses. In addition, antigen-specific regulatory T cells can produce anti-inflammatory cytokines after encountering a previously administered autoantigen (60, 61). There is evidence that TH1 type responses to MBP are associated with worse neurological outcome following experimental stroke (60, 62, 63). In one study, the adoptive transfer of MBP specific TH1 (+) or TH17 (+) cells into naïve recipient mice resulted in a worse neurological outcome (63). The adoptive transfer of splenocytes reactive for MOG into immunodeficient MCAO mice led to their migration into the ischemic hemispheres and resulted in an increased infarct volume (64). However, TH1 responses to antigen stimulation with MBP following 1 month after experimental MCAO were low and animals were more likely to develop a TH2/TH3 type response (60). Treatment with the pathogenic lipopolysaccharide (LPS) to stimulate a systemic inflammatory response, showed an increased sensitization to MBP with an increased TH1-response and more severe neurologic deficits and brain atrophy at 1 month after MCAO. This suggested that a systemic inflammation after stroke could lead to a detrimental autoimmune response (60, 65). LPS also induced the expression of MHC II molecules on microglia required for lymphocyte activation which are usually not expressed in the CNS (66). In a more recent study of the same group, which investigated autoimmune responses to MBP after stroke following immunization with MBP, again only 1/4–1/3 of the animals had a TH1 or TH17 response to MBP. Those with a response were also associated with worse outcome at 1 month (67). The detrimental effect was observed shortly after experimental ischemia. Animals that were sensitized to MBP prior to stroke had a sharply increased mortality within 24 h, whereas induction of oral tolerance to MBP resulted in significantly reduced infarct sizes at 24 h and 96 h after ischemia (68). MCAO animals who received splenocytes from MBP tolerized donors showed a decrease in infarct volume (69). Animals who were tolerized to MBP before MCAO induction and LPS treatment were less likely to develop a TH1 response to MBP than ovalbumin (OVA) tolerized animals and had an increased probability of a TH3 or TREG response together with smaller infarct sizes after 1 months (55). Similarly, mice who were previously nasally tolerized to MOG showed a significant shift from the proinflammatory cytokine IFN-γ to the anti-inflammatory cytokine IL-10 after MCAO induction (61). Adoptive transfer of CD4+ T cells from nasally tolerized mice to wild-type mice showed a significant reduction in infarct size. This effect could not be observed in IL-10 deficient mice (61). Treatment with a neuroantigen-specific peptide such as recombinant T cell receptor ligands (RTL), linked to myelin peptide (MOG), reduced lesion size and inhibited the accumulation of inflammatory cells, presumably by inducing tolerance to MOG (70). RTL specifically targeting myelin-specific T cells can modulate their characteristics to anti-inflammatory cells that secrete IL-10 (71).

Similar observations have also been made in clinical studies. In a study with 114 patients of ischemic stroke, a strong TH1 response to MBP at 90 days was associated with worse outcome and was more likely to develop in patients with severe stroke and post-stroke infection (72). The degree of the TH1 response however decreased over time, pointing to the possibility of immunoregulatory mechanisms (73). Recently, in brain slices from 5 patients with acute middle cerebral artery ischemic stroke, who died within 7–10 days after stroke onset, T cells, including MOG-specific CD4+ T cells were found in the infarct and peri-infarct area close to ischemic neurons (34). In a cohort of 28 patients with milder ischemic stroke, frequencies of MOG and MBP-specific IFN-γ responses, that were measured in peripheral blood, were increased early within the first week after stroke and decreased at 3 months. Naïve CD4+ and CD8+ T cells were also decreased within the first days after stroke, which was attributed in the context of stroke-induced immunosuppression (SIDS) to a possible adaptive mechanism, preventing long-term autoimmune responses (74). Accordingly, blockade of SIDS in transgenic mice with the CD4+ MOG T cell receptor, resulted in increased antigen specific TH1 responses at 14 days after MCAO (75).

Repetitive mucosal tolerance with low doses of antigen is known to generate regulatory T cells and suppress EAE by inducing peripheral tolerance (76). In one study, repetitive mucosal tolerance to E-selectin, a glycoprotein adhesion molecule expressed on activated endothelial cells after stroke, suppressed TH1-mediated delayed-type hypersensitivity (DTH) reaction to E-selectin, and increased IL-10 secretion in adoptively transferred splenocytes from tolerized animals. Reduced infarct sizes could be detected as early as 6 and 48 h after MCAO (54). In another study of mucosal tolerance to E-selectin, an increased neurogenesis together with increased Tregs in the cervical lymph nodes and peri-infarct region were found after pMCAO together with an improved behavioral recovery at 2–4 weeks (56).

Taken together, antigen specific tolerance can be used in some animal models to lessen stroke damage. These experiments show tolerization, however the exact mechanisms are not fully understood. This could be T cell modifying factors, but also possibly T cell independent.

B CELLS: AUTOANTIBODIES IN STROKE

B lymphocytes originate in the bone marrow and differentiate in the spleen. B cell activation occurs in the secondary lymphoid organs such as the spleen or lymph nodes and requires the recognition of antigen by B cell receptors (BCR) and often signaling by T helper cells. B cells progress through an antigen-independent combinatorial rearrangement of the variable (V), diversity (D) and joining (J) gene segments. This leads to the development of a vast repertoire of VDJ segments encoding the BCR, capable of recognizing a huge diversity of antigens (77). Autoreactive B cells initially constitute 75% of immature B cells and undergo several selection processes in the bone marrow

and spleen to establish self-tolerance (78). Negative selection occurs in B cells that recognize self-antigen with their B cell receptor (BCR); they undergo apoptosis, receptor editing, or anergy leading to a state of central tolerance lacking pathological autoreactivity. However, these self-tolerance mechanisms fail to delete all B cells reactive to brain antigens. Brain-reactive autoantibodies can be detected in 92% of human sera (79). B cells can also acquire self-reactivity through somatic hypermutation (SMH). This process follows antigen-dependent activation and allows through affinity maturation, the diversification of B cell receptors through mutations within the variable gene regions of immunoglobulins (80). B cells can also change their constant region of their immunoglobulin heavy (IgH) chain referred to as IgH class switch recombination (CSR). Naive mature B cells produce both IgM and IgD, which are the first two heavy chain segment in the immunoglobulin locus. After activation and T cell dependent cytokine signaling, they undergo antibody class switching to produce IgG, IgA, or IgE antibodies (80). IgM antibodies are the first antibodies to respond to antigen exposure and are associated with primary immune response. IgG are associated with the secondary immune response and represent the vast majority of serum antibodies found in humans. They are major components of humoral immunity involved in controlling infections and neutralizing pathogens and toxins.

The production of autoantibodies requires the antigen-dependent activation of B cells following ischemia. Brain antigens need to bind to the BCR of an autoreactive B cell. They are then taken up in to the B cells by receptor-mediated endocytosis, degraded, and presented by MHC-II molecules on the cell membrane (81). In the case of T cell dependent activation, autoreactive T cells activated by the same antigen, would need to bind to MHC-II through their T cell receptor (TCR) followed by expression of co-stimulatory factors and cytokines which promote B cell proliferation, somatic hypermutation and class switching. The expression of costimulatory molecules can be increased following infection (60) and the release of damage-associated-molecular patterns (DAMPs) after stroke (82). In the case of T cell independent activation, B cells are stimulated by antigens such as pathogen associated molecular patterns (PAMPs), like bacterial polysaccharides or extensive cross-linking of BCRs to repeated epitopes of an antigen. Pathogenic antigens can be increased because of post-stroke infection which is a common occurrence due to stroke-induced immunosuppression (83, 84). The pathway of brain antigen release, as described in the T cell section above, would allow antigen-dependent activation to take place in the CNS and the secondary lymphoid tissue such as the cervical lymph nodes (5).

There is evidence of an immunoglobulin production in the cerebrospinal fluid (CSF) of patients with cerebrovascular disease (85, 86). In the CSF of 318 stroke patients IgG, IgM, and IgA immunoglobulin synthesis was significantly elevated in 24.8% of patients and 17.9% had CSF-specific oligoclonal IgG band, compared to 2.5% in age- and sex-matched controls (87). The absence of oligoclonal bands in the serum of stroke patients suggests an additional autoimmune B cell activation in the CNS outside of the cervical lymph nodes in the weeks after stroke (83). Activated B lymphocytes producing IgG, IgM, and IgA could be found in the ischemic mouse brain adjacent to the lesion beginning 2 weeks following stroke with extensive infiltration at 7 weeks. The B cells were surrounded by cells expressing the dendritic cell marker CD11c, which suggests antigen-presentation. B cells that were clustered with T lymphocytes were compartmentalized, suggestive of structures similar to ectopic B cell follicle with germinal centers (40, 83). In autopsy brain tissue of 21 stroke patients, more B cells, and IgG positive antibodies were found than in controls (40). In other studies, elevated autoantibodies to brain specific neurofilaments (NF) were found in the serum of stroke patients in the first 6 months, whereas antibodies to the ubiquitous antigen, cardiolipin (CL) were not elevated (88).

There is also evidence of autoantibodies following stroke that bind to the N-methyl-D-aspartate receptor (NMDAR). The condition of Anti-NMDA-receptor encephalitis is mediated by autoantibodies that target the GluN1 (NR1) subunit of NMDA receptors in brain (89). The serum levels of autoantibodies to the GluN2 (NR2) subtype of NMDA receptors were elevated within 3 h and peaked at 10–12 h in patients after stroke or TIA (90). Patients with elevated GluN2 (NR2) antibodies before cardiac surgery were 18 times more likely to experience a postoperative neurological event such as confusion, TIA or stroke than following a negative NR2 antibody test (91). This could be due to an already compromised cerebrovascular circulation and ongoing silent ischemia. Accordingly, the presence of serum autoantibodies to the NMDA GluN2 (NR2) receptor showed a correlation with risk factors for stroke such as diabetes, hypertension or recent stroke/TIA (92). In another study, antibodies against the GluN1-S2 subunit of the NMDA receptor were found in the serum of 44% of 48 stroke patients compared to 3% in age matched healthy controls within 48 h of stroke onset. The presence of these antibodies was associated with worse clinical state on presentation, larger infarcts and cortical involvement (93). In contrast, increased immunoreactivity to the NMDA GluN2 (NR2) subunit in lymphoid tissue was associated with smaller infarcts within the first week of stroke (5). The early detection of increased autoantibodies levels within hours after stroke onset is suggestive of a rapid amplification of naturally occurring autoantibodies following stroke related antigen exposure (94). The role of naturally occurring IgM antibodies to annexin IV and a subset of phospholipids, was assessed by Elvington et al. (95). Ischemia reperfusion injury was mediated through the postischemic binding of natural IgMs to neoepitopes in the infarct area with complement activation and a proinflammatory phenotype in a T cell independent process.

B CELLS–BENEFICIAL OR DETRIMENTAL?

In order to assess the beneficial or detrimental effect of B cells, the time after stroke and the different B cells subsets need to be considered. Regulatory B cells constitute only 0.5–0.7% of CD19+ B cells (96) and have been associated with protective effects following stroke. B cell deficient mice that received B cells from IL $10^{-/-}$ mice had larger infarct volumes,

more severe functional deficits, higher mortality and increased numbers of activated T cells, macrophages, microglial cells, and neutrophils in the affected brain 48 h after stroke induction in the MCAO model (97). B cell deficient mice that received IL-10+ B cells had reduced infarcts, fewer infiltrating cells and a significant increase in regulatory T cells (98). The intrastriatal injection of cells, containing all $CD19^+$ B cell subsets, into B cell deficient mice reduced infarct volumes 48 h after stroke onset. This would suggest a strong role for the default mixture of B cells to favor immunoregulation over inflammation early after experimental stroke (96). Conversely, (8) found B cells to play only a minor role in acute ischemia and (40) found no difference in infarct volumes at 72 h in B cell deficient mice. In the same study, a delayed detrimental role of B cells was discovered, with cognitive impairment within 7 weeks of stroke as a possible cause of post-stroke dementia. Delayed cognitive impairment was circumvented in B cell deficient mice and wild-type mice by B cell depletion with an antibody 5 days after stroke (40). In a cohort of 58 stroke patients, elevated titers of serum antibodies to MBP were associated with cognitive decline in the year after stroke (99). In other models, an early protective effect of regulatory B cells and a delayed detrimental effect of B cells have been found. In EAE, regulatory B cells have only shown a protective anti-inflammatory role during early EAE induction. On the other hand, B cell depletion during disease expansion reduced the infiltration of encephalitogenic T cells within the CNS and draining lymph nodes and inhibited MOG-specific T cell expansion, dramatically reducing disease symptoms (100). In experimental brain injury models, such as spinal cord lesions, B cell deficient mice have shown improved locomotor function, and reduced lesion pathology compared with wild-type mice that had antibody producing B cells and IgGs in their CSF or injured spinal cord (101, 102).

Taken together there is evidence for naturally occurring brain-reactive antibodies that escape tolerance mechanisms prior to stroke onset. Antibody titers and their presence are dramatically increased in the first hours and days following stroke. This would suggest a rapid augmentation of naturally occurring autoantibodies upon encountering increased levels of brain antigen. The delayed detection of autoantibodies and clusters of B cells and T cells in brain lesions could indicate a stroke-related generation of novel autoantibodies. There are conflicting results regarding the detrimental or beneficial effects of brain-reactive autoantibodies in the acute phase of stroke and further studies are needed. Finally, regulatory B cells seem to be protective early in stroke, while delayed detrimental effects have been shown for B cells.

BRAIN TRAUMA

Traumatic brain injury (TBI) involves a primary injury with multifocal damage to axons, glia, dendrites, and a microvasculopathy with microbleeds, endothelial damage, and breach of the blood brain barrier (BBB) (103, 104). Similar to stroke, it is followed by a secondary injury involving an inflammatory reaction with pro-and anti-inflammatory cytokines, microglial activation, invasion of peripheral neutrophils, lymphocytes and macrophages (105), damaging levels of glutamate (106), and free radicals (107, 108).

Biomarkers that have been identified in human and animal biofluids after TBI include, glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), neuron specific enolase (NSE), glia calcium-binding protein S100B, ubiquitin carboxyl hydrolase-like 1 (UCHL1), αII-spectrin fragments, and neurofilament proteins (109–115). The release of these brain proteins from injured cells into the bloodstream and the aforementioned neuroinflammatory response can trigger an autoimmune response with the generation of autoantibodies.

AUTOANTIBODIES IN TBI

The presence of long-term hypopituitarism after TBI, most frequently growth hormone (GH) deficiency, has led researchers to investigate anti-hypothalamus antibodies (AHA), and anti-pituitary antibodies (APA), showing significant associations between these antibodies and head trauma (116, 117). High titers of APA and AHA in boxers were associated with hypopituitarism even after 5 years following TBI (118). The pathophysiological pathway of hypopituitarism and the role of autoimmunity in changes of hormone levels remains to be investigated.

S100B is a calcium-binding protein, present in perivascular astrocytes, but also in the CNS as an astrocyte-specific protein. S100B increases in the CSF and serum in correlation to the severity of traumatic brain injuries within the first hours to days (115, 119, 120). In football players experiencing repetitive sub-concussive head hits, increased levels of S100B could be detected following individual games. Players with the most significant elevations of S100B had the highest titer of serum S100B autoantibodies at the end of a season. The level of S100B autoantibodies predicted MRI abnormalities and correlated with cognitive changes (121). S100B leakage into serum, could therefore have contributed to the development of autoantibodies after one season. MBP autoantibodies have been measured in the CSF of patients with a strong correlation to the severity of injury, which was measured by the Glasgow Coma Scale (GCS) (122). GFAP is an intermediate filament protein, predominantly found in the cytoskeleton of astrocytes. GFAP autoantibody levels increased by 7 days after TBI in humans (123) and were also found at a chronic time point 6 months post-injury (124). There were also higher plasma autoantibody levels against GFAP in patients with acute TBI and previous exposure to TBI compared to those without a previous history of TBI (124). Serum levels of autoantibodies against AMPA glutamate receptors (GluR1 subunit) and NMDA glutamate receptor (NR2A subunit) were examined in the serum from 60 children (7–16 years) with chronic posttraumatic headache at 6 and 12 months following trauma. Antibody titers to AMPA and largely NMDA were found to be higher when neurological symptoms persisted in children, which according to the authors was suggestive of hypoxic brain lesions, and evidence of hyperstimulation of NMDA glutamate receptors with greater permeability of the blood-brain-barrier (125).

Taken together, elevated levels of brain-reactive autoantibodies can also be found following clinical TBI, in many cases correlating to the severity of injury and frequency of head impacts. The presence of autoantibodies does not provide evidence for an autoimmune disease. The pathogenic potential of brain-reactive antibodies, that could enter the brain through a compromised BBB remains to be explored.

ADAPTIVE IMMUNITY IN TBI

The specific role of neuroinflammation and autoantibodies in the recovery and neuropathology of TBI patients remains unclear. TBI induced neuropathology with accumulation of proteins such as amyloid beta (Aβ) has been associated with an increased risk of neurological diseases such as Alzheimer's disease (AD) and chronic traumatic encephalopathy (CTE) (126). The question whether chronic neuroinflammation causes this neuropathology or if it is a response to abnormal protein accumulation is not understood (105). In a recent study, concussion in adolescence was associated with an increased risk of multiple sclerosis (127). Experimental evidence points to a link between injury to the nervous system and myelin-antigen-specific T cells, that have been shown to result in encephalomyelitis, if the genetic predisposition of an autoimmune disease is present (128). In one study reactive microglia were found in 28% of patients with traumatic brain injury with survival of >1 years and were linked to white matter degeneration and thinning of the corpus callosum (129). In another study, microglial activation was associated with more severe cognitive impairment years after trauma (130). Early T cell infiltration, peaking as early as 3–24 h after experimental TBI has been described in previous studies (131, 132). In another experimental study intracerebral injection of ovalbumin led to the infiltration and accumulation of ovalbumin-specific CD8+ T cells at sites of the cognate antigen. Following TBI, these activated antigen-specific CD8+ T cells were also attracted to sites of traumatic injury. This process could not be reduced by blocking CD154, which is presumed to be important for antigen-specific T cell activation, pointing to a presumably antigen-independent attraction to injury (133). In an experimental study of weight-drop induced closed head injury, $Rag1^{-/-}$ mice deficient of B and T lymphocytes, showed no difference regarding neurological and histological outcome and inflammatory mediators for up to 7 days following injury, compared with wild-type mice (134). In a similar study of weight-drop TBI, FTY720 was used to significantly reduce the numbers of circulating and brain infiltrating lymphocytes. There was no difference between BBB integrity, lesion size or functional outcome at 24 up to 7 days compared to untreated mice (135). This suggests that early T cell recruitment, which would presumably be antigen-independent, plays no role in early TBI up to 1 week. MBP autoreactive T cells were found in 40% of 10 patients 10 days after TBI using proliferation assays, but with no correlation to injury volume or severity in one study (136). The role of lymphocytes in TBI mediated inflammation and recovery, the question of antigen-specificity and activation pathways, especially in chronic models of TBI remain to be investigated.

ADAPTIVE IMMUNITY IN OTHER CNS LESION MODELS

In others models of injury to the CNS, there is evidence for a protective role of specific T cells. In optic nerve injury passively transferred T cells have been shown to accumulate at the injury site between 3 and 21 days irrespective of their antigen-specificity (MBP vs. ovalbumin) (137). The transfer of T cells specific to MBP to mice with optic nerve injury however promoted recovery, while this effect could not be observed with activated T cells to other non CNS-antigens (e.g., ovalbumin) (138). Mice that were actively immunized with myelin associated peptides such as MOG before injury to the optic nerve also had less degeneration of retinal ganglion cells (139). Similarly inducing tolerance to myelin proteins in neonates decreased the ability to a protective T cell mediated immune response to injury of myelinated axons in the CNS (140). Autoreactive T cells to myelin proteins such as MBP have also been found significantly proliferated in clinical and experimental spinal cord injury (SCI) (141–144) together with increased levels of serum autoantibodies against MAG and GM1 ganglioside in patients (145). In a model of facial nerve injury an expansion of myelin-antigen-specific T cells secreting IFN-y in cervical lymph nodes could be seen after 8 days (146). The transfer of anti-MBP T cells or the active immunization with MBP at the time and 1 week after contusion in an experimental rat model of SCI in both cases resulted in a significantly better functional outcome (147). In contrast, endogenous MBP-reactive lymphocytes, activated by experimental traumatic SCI contributed to tissue injury and impaired functional recovery (148). In a model of MBP (63–68) peptide induced EAE, encephalitogenic T cells using the TCR-$BV8S2^{+}$ had a much higher proinflammatory cytokine to neurotrophin ratio than lymphoid cells bearing other TCRs. This would suggest that neuroprotection could be mediated by other non-MPB peptide-specific lymphoid cells (149, 150).

The question of the neuroprotective role of T cells and their antigen-specific activation was further addressed in a study with optic nerve crush injury and spinal cord injury. CD4+ T cells were activated independently of MHC II via damage-associated molecular mediators from CNS tissue that induced a population of neuroprotective IL-4 producing T cells. Recovery was promoted via neuronal IL-4 receptors that potentiated neurotrophin signaling (151). This suggests that antigen-independent pathways also play a role in neuroprotection after CNS injury. The question whether this protective mechanism is later enhanced by an autoimmune T cell response or if T cell infiltration could be increased through vaccination with an appropriate CNS self-antigen remains to be explored (139).

Recently, it was shown, that autoantibodies directed against different nervous system and systemic self-antigens, that increased within 5 days of human SCI, were already present in healthy subjects (152). Similar to stroke, this is an indication of a rapid augmentation of pre-existing naturally occurring autoantibodies. The role of autoreactive B cells has been investigated in SCI-induced autoimmunity (101, 102, 153). SCI induces B cell proliferation, activation, and differentiation into IgG+ plasma cells. The formation of large B cell clusters

expressing MHC II surrounded by CD3+ T cells and increased IgG synthesis after SCI resembles lymphoid follicles with B cell and T cell interactions (101). There is a delayed accumulation of B cells in the CSF and injured spinal cord with large deposits of antibody at sites of axon pathology and demyelination (102). Injection of circulating IgG antibodies purified from SCI mice in the spinal cord of uninjured animals causes consistent paralysis and pathology and B cell deficient mice have smaller lesions and better functional outcome after SCI (102).

CONCLUSION

Stroke and Traumatic Brain Injury (TBI) cause the release of brain-derived antigens into the circulation. In stroke, the detection of autoreactive T cells and the presence of autoantibodies to brain antigens suggests the possibility of autoimmunity. Inducing immune tolerance to brain antigens prior to experimental stroke shows mostly beneficial effects. Increased immunoreactivity to brain antigens and brain-reactive antibodies is associated with mostly detrimental effects, but some beneficial effects have also been shown. The early immunoreactivity to brain-antigens and detection of elevated levels of autoantibodies within hours to days after stroke onset, suggests the expansion of naturally occurring autoantibodies and pre-existing self-reactive T cells. The delayed presence of B cell and T cell clusters in stroke lesions with antibody production correlating to cognitive decline could be an indication of a detrimental adaptive immune response. The proinflammatory microenvironment and infections following stroke could favor autoimmune responses to brain antigens breaking tolerance mechanisms. In TBI, the levels of brain antigens and brain-reactive antibodies correlate with the severity of stroke and frequency of head impacts in most studies. If these antibodies are pathogenic, remains to be further explored. There is little evidence for the early role of T cells in early TBI and their long-term effects need to be investigated. In optic nerve crush injury and spinal cord lesion models, autoreactive T cells show protective effects, while antibody producing B cells exacerbate spinal cord injury. Taken together, the existing data in favor of autoimmunity remains inconclusive. The effect of self-reactive autoantibodies is possibly small compared to other detrimental effects of the lesion itself and the induced local inflammation. Further studies are needed to understand the role of autoimmunity after brain lesions, investigating the generation, function and pathway of autoantibodies and the extent of their protective or detrimental role, with possible therapeutic implications.

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REFERENCES

1. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. *Nat Med.* (2011) 17:796–808. doi: 10.1038/nm.2399
2. Corps KN, Roth TL, McGavern DB. Inflammation and neuroprotection in traumatic brain injury. *JAMA Neurol.* (2015) 72:355–62. doi: 10.1001/jamaneurol.2014.3558
3. Gelderblom M, Leypoldt F, Steinbach K, Behrens D, Choe CU, Siler DA, et al. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. *Stroke.* (2009) 40:1849–57. doi: 10.1161/STROKEAHA.108.534503
4. Das M, Mohapatra S, Mohapatra SS. New perspectives on central and peripheral immune responses to acute traumatic brain injury. *J Neuroinflamm.* (2012) 9:236. doi: 10.1186/1742-2094-9-236
5. Planas AM, Gomez-Choco M, Urra X, Gorina R, Caballero M, Chamorro A. Brain-derived antigens in lymphoid tissue of patients with acute stroke. *J Immunol.* (2012) 188:2156–63. doi: 10.4049/jimmunol.1102289
6. Wang P, Zheng SG. Regulatory T cells and B cells: implication on autoimmune diseases. *Int J Clin Exp Pathol.* (2013) 6:2668–74.
7. Wang L, Wang FS, Gershwin ME. Human autoimmune diseases: a comprehensive update. *J Intern Med.* (2015) 278:369–95. doi: 10.1111/joim.12395
8. Kleinschnitz C, Schwab N, Kraft P, Hagedorn I, Dreykluft A, Schwarz T, et al. Early detrimental T-cell effects in experimental cerebral ischemia are neither related to adaptive immunity nor thrombus formation. *Blood.* (2010) 115:3835–42. doi: 10.1182/blood-2009-10-249078
9. Clarkson BD, Ling C, Shi Y, Harris MG, Rayasam A, Sun D, et al. T cell-derived interleukin (IL)-21 promotes brain injury following stroke in mice. *J Exp Med.* (2014) 211:595–604. doi: 10.1084/jem.20131377
10. Brait VH, Arumugam TV, Drummond GR, Sobey CG. Importance of T lymphocytes in brain injury, immunodeficiency, and recovery after cerebral ischemia. *J Cerebr Blood Flow Metab.* (2012) 32:598–611. doi: 10.1038/jcbfm.2012.6
11. Ludewig P, Sedlacik J, Gelderblom M, Bernreuther C, Korkusuz Y, Wagener C, et al. Carcinoembryonic antigen-related cell adhesion molecule 1 inhibits MMP-9-mediated blood-brain-barrier breakdown in a mouse model for ischemic stroke. *Circ Res.* (2013) 113:1013–22. doi: 10.1161/CIRCRESAHA.113.301207
12. Jiang X, Andjelkovic AV, Zhu L, Yang T, Bennett MVL, Chen J, et al. Blood-brain barrier dysfunction and recovery after ischemic stroke. *Prog Neurobiol.* (2018) 163-164:144–71. doi: 10.1016/j.pneurobio.2017.10.001
13. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Sci Transl Med.* (2012) 4:147ra11. doi: 10.1126/scitranslmed.3003748
14. Ma Q, Ineichen BV, Detmar M, Proulx ST. Outflow of cerebrospinal fluid is predominantly through lymphatic vessels and is reduced in aged mice. *Nat Commun.* (2017) 8:1434. doi: 10.1038/s41467-017-01484-6
15. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, et al. Structural and functional features of central nervous system lymphatic vessels. *Nature.* (2015) 523:337. doi: 10.1038/nature14432
16. Verheggen ICM, Van Boxtel MPJ, Verhey FRJ, Jansen JFA, Backes WH. Interaction between blood-brain barrier and glymphatic system in solute clearance. *Neurosci Biobehav Rev.* (2018) 90:26–33. doi: 10.1016/j.neubiorev.2018.03.028
17. Felger JC, Abe T, Kaunzner UW, Gottfried-Blackmore A, Gal-Toth J, McEwen BS, et al. Brain dendritic cells in ischemic stroke: time course, activation state, and origin. *Brain Behav Immun.* (2010) 24:724–37. doi: 10.1016/j.bbi.2009.11.002
18. Ludewig P, Gallizioli M, Urra X, Behr S, Brait VH, Gelderblom M, et al. Dendritic cells in brain diseases. *Biochim Biophys Acta.* (2016) 1862:352–67. doi: 10.1016/j.bbadis.2015.11.003

19. Liesz A, Karcher S, Veltkamp R. Spectratype analysis of clonal T cell expansion in murine experimental stroke. *J Neuroimmunol.* (2013) 257:46–52. doi: 10.1016/j.jneuroim.2013.01.013
20. de Vos AF, van Meurs M, Brok HP, Boven LA, Hintzen RQ, van der Valk P, et al. Transfer of central nervous system autoantigens and presentation in secondary lymphoid organs. *J Immunol.* (2002) 169:5415–23. doi: 10.4049/jimmunol.169.10.5415
21. Bartholomaus I, Kawakami N, Odoardi F, Schlager C, Miljkovic D, Ellwart JW, et al. Effector T cell interactions with meningeal vascular structures in nascent autoimmune CNS lesions. *Nature.* (2009) 462:94–8. doi: 10.1038/nature08478
22. Miró-Mur F, Urra X, Gallizioli M, Chamorro A, Planas AM. Antigen presentation after stroke. *Neurotherapeutics.*(2016) 13:719–28. doi: 10.1007/s13311-016-0469-8
23. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature.* (1998) 392:245–52. doi: 10.1038/32588
24. Garza KM, Chan SM, Suri R, Nguyen LT, Odermatt B, Schoenberger SP, et al. Role of antigen-presenting cells in mediating tolerance and autoimmunity. *J Exp Med.* (2000) 191:2021–7. doi: 10.1084/jem.191.11.2021
25. Benakis C, Llovera G, Liesz A. The meningeal and choroidal infiltration routes for leukocytes in stroke. *Ther Adv Neurol Disord.* 11:1756286418783708–1756286418783708. doi: 10.1177/1756286418783708
26. Audiger C, Rahman MJ, Yun TJ, Tarbell KV, Lesage S. The importance of dendritic cells in maintaining immune tolerance. *J Immunol.* (2017) 198:2223–31. doi: 10.4049/jimmunol.1601629
27. Gelderblom M, Gallizioli M, Ludewig P, Thom V, Arunachalam P, Rissiek B, et al. IL-23 (Interleukin-23)-producing conventional dendritic cells control the detrimental IL-17 (interleukin-17) response in Stroke. *Stroke.* (2018) 49:155–64. doi: 10.1161/STROKEAHA.117.019101
28. Almolda B, Gonzalez B, Castellano B. Antigen presentation in EAE: role of microglia, macrophages and dendritic cells. *Front Biosci.* (2011) 16:1157–71. doi: 10.2741/3781
29. Nuyts AH, Lee WP, Bashir-Dar R, Berneman ZN, Cools N. Dendritic cells in multiple sclerosis: key players in the immunopathogenesis, key players for new cellular immunotherapies? *Mult Scler.* (2013) 19:995–1002. doi: 10.1177/1352458512473189
30. Bailey SL, Schreiner B, McMahon EJ, Miller SD. CNS myeloid DCs presenting endogenous myelin peptides 'preferentially' polarize CD4+ T(H)-17 cells in relapsing EAE. *Nat Immunol.* (2007) 8:172–80. doi: 10.1038/ni1430
31. Gülke E, Gelderblom M, Magnus T. Danger signals in stroke and their role on microglia activation after ischemia. *Ther Adv Neurol Disord.* 11:1756286418774254. doi: 10.1177/1756286418774254
32. Luo C, Jian C, Liao Y, Huang Q, Wu Y, Liu X, et al. The role of microglia in multiple sclerosis. *Neuropsychiatr Dis Treat.* (2017) 13:1661–7. doi: 10.2147/NDT.S140634
33. Almolda B, Gonzalez B, Castellano B. Activated microglial cells acquire an immature dendritic cell phenotype and may terminate the immune response in an acute model of EAE. *J Neuroimmunol.* (2010) 223:39–54. doi: 10.1016/j.jneuroim.2010.03.021
34. Jin WN, Gonzales R, Feng Y, Wood K, Chai Z, Dong JF, et al. Brain ischemia induces diversified neuroantigen-specific T-cell responses that exacerbate brain injury. *Stroke.* (2018) 49:1471–8. doi: 10.1161/STROKEAHA.118.020203
35. Gronberg NV, Johansen FF, Kristiansen U, Hasseldam H. Leukocyte infiltration in experimental stroke. *J Neuroinflammation.* (2013) 10:115. doi: 10.1186/1742-2094-10-115
36. Chu HX, Kim HA, Lee S, Moore JP, Chan CT, Vinh A, et al. Immune cell infiltration in malignant middle cerebral artery infarction: comparison with transient cerebral ischemia. *J Cereb Blood Flow Metab.* (2014) 34:450–9. doi: 10.1038/jcbfm.2013.217
37. Mracsko E, Javidi E, Na SY, Kahn A, Liesz A, Veltkamp R. Leukocyte invasion of the brain after experimental intracerebral hemorrhage in mice. *Stroke.* (2014) 45:2107–14. doi: 10.1161/STROKEAHA.114.005801
38. Stevens SL, Bao J, Hollis J, Lessov NS, Clark WM, Stenzel-Poore MP. The use of flow cytometry to evaluate temporal changes in inflammatory cells following focal cerebral ischemia in mice. *Brain Res.* (2002) 932:110–9. doi: 10.1016/S0006-8993(02)02292-8
39. Liesz A, Zhou W, Mracsk,ó É, Karcher S, Bauer H, Schwarting S, et al. Inhibition of lymphocyte trafficking shields the brain against deleterious neuroinflammation after stroke. *Brain.* (2011) 134:704–720. doi: 10.1093/brain/awr008
40. Doyle KP, Quach LN, Sole M, Axtell RC, Nguyen TV, Soler-Llavina GJ, et al. B-lymphocyte-mediated delayed cognitive impairment following stroke. *J Neurosci.* (2015) 35:2133–45. doi: 10.1523/JNEUROSCI.4098-14.2015
41. Gill D, Veltkamp R. Dynamics of T cell responses after stroke. *Curr Opin Pharmacol.* (2016) 26:26–32. doi: 10.1016/j.coph.2015.09.009
42. Yilmaz G, Arumugam TV, Stokes KY, Granger DN. Role of T lymphocytes and interferon-gamma in ischemic stroke. *Circulation.* (2006) 113:2105–12. doi: 10.1161/CIRCULATIONAHA.105.593046
43. Liesz A, Suri-Payer E, Veltkamp C, Doerr H, Sommer C, Rivest S, et al. Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. *Nat Med.* (2009) 15:192–9. doi: 10.1038/nm.1927 ·
44. Shichita T, Sugiyama Y, Ooboshi H, Sugimori H, Nakagawa R, Takada I, et al. Pivotal role of cerebral interleukin-17–producing γδT cells in the delayed phase of ischemic brain injury. *Nat Med.* (2009) 15:946. doi: 10.1038/nm.1999
45. Mracsko E, Liesz A, Stojanovic A, Lou WP, Osswald M, Zhou W, et al. Antigen dependently activated cluster of differentiation 8-positive T cells cause perforin-mediated neurotoxicity in experimental stroke. *J Neurosci.* (2014) 34:16784–95. doi: 10.1523/JNEUROSCI.1867-14.2014
46. Ortega SB, Noorbhai I, Poinsatte K, Kong X, Anderson A, Monson NL, et al. Stroke induces a rapid adaptive autoimmune response to novel neuronal antigens. *Discov Med.* (2015) 19:381–92.
47. Dominguez-Villar M, Hafler DA. Regulatory T cells in autoimmune disease. *Nat Immunol.* (2018) 19:665–73. doi: 10.1038/s41590-018-0120-4
48. Liesz A, Suri-Payer E, Veltkamp C, Doerr H, Sommer C, Rivest S, et al. Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. *Nat Med.* (2009) 15:192–9. doi: 10.1038/nm.1927
49. Stubbe T, Ebner F, Richter D, Engel O, Randolf Engel O, Klehmet J, et al. Regulatory T cells accumulate and proliferate in the ischemic hemisphere for up to 30 days after MCAO. *J Cereb Blood Flow Metab.* (2013) 33:37–47. doi: 10.1038/jcbfm.2012.128
50. Liesz A, Hu X, Kleinschnitz C, Offner H. Functional role of regulatory lymphocytes in stroke: facts and controversies. *Stroke.* (2015) 46:1422–30. doi: 10.1161/STROKEAHA.114.008608
51. Offner H, Subramanian S, Parker SM, Wang C, Afentoulis ME, Lewis A, et al. Splenic atrophy in experimental stroke is accompanied by increased regulatory T cells and circulating macrophages. *J Immunol.* (2006) 176:6523–31. doi: 10.4049/jimmunol.176.11.6523
52. Kleinschnitz C, Kraft P, Dreykluft A, Hagedorn I, Göbel K, Schuhmann MK, et al. Regulatory T cells are strong promoters of acute ischemic stroke in mice by inducing dysfunction of the cerebral microvasculature. *Blood.* (2013) 121:679–91. doi: 10.1182/blood-2012-04-426734
53. Ito M, Komai K, Mise-Omata S, Iizuka-Koga M, Noguchi Y, Kondo T, et al. Brain regulatory T cells suppress astrogliosis and potentiate neurological recovery. *Nature.* (2019) 565:246–50. doi: 10.1038/s41586-018-0824-5
54. Chen Y, Ruetzler C, Pandipati S, Spatz M, McCarron RM, Becker K, et al. Mucosal tolerance to E-selectin provides cell-mediated protection against ischemic brain injury. *Proc Natl Acad Sci USA.* (2003) 100:15107–12. doi: 10.1073/pnas.2436538100
55. Gee JM, Kalil A, Thullbery M, Becker KJ. Induction of immunologic tolerance to myelin basic protein prevents central nervous system autoimmunity and improves outcome after stroke. *Stroke.* (2008) 39:1575–82. doi: 10.1161/STROKEAHA.107.501486
56. Ishibashi S, Maric D, Mou Y, Ohtani R, Ruetzler C, Hallenbeck JM. Mucosal tolerance to E-selectin promotes the survival of newly generated neuroblasts via regulatory T-cell induction after stroke in spontaneously hypertensive rats. *J Cereb Blood Flow Metab.* (2009) 29:606–20. doi: 10.1038/jcbfm.2008.153
57. Wang WZ, Olsson T, Kostulas V, Hojeberg B, Ekre HP, Link H. Myelin antigen reactive T cells in cerebrovascular diseases. *Clin Exp Immunol.* (1992) 88:157–62. doi: 10.1111/j.1365-2249.1992.tb03056.x
58. Jauch EC, Lindsell C, Broderick J, Fagan SC, Tilley BC, Levine SR. Association of serial biochemical markers with acute ischemic stroke: the national institute of neurological disorders and stroke recombinant

tissue plasminogen activator stroke study. *Stroke.* (2006) 37:2508–13. doi: 10.1161/01.STR.0000242290.01174.9e
59. Walsh KP, Mills KH. Dendritic cells and other innate determinants of T helper cell polarisation. *Trends Immunol.* (2013) 34:521–30. doi: 10.1016/j.it.2013.07.006
60. Becker KJ, Kindrick DL, Lester MP, Shea C, Ye ZC. Sensitization to brain antigens after stroke is augmented by lipopolysaccharide. *J Cereb Blood Flow Metab.* (2005) 25:1634–44. doi: 10.1038/sj.jcbfm.9600160
61. Frenkel D, Huang Z, Maron R, Koldzic DN, Moskowitz MA, Weiner HL. Neuroprotection by IL-10-producing MOG CD4+ T cells following ischemic stroke. *J Neurol Sci.* (2005) 233:125–32. doi: 10.1016/j.jns.2005.03.022
62. Zierath D, Thullbery M, Hadwin J, Gee JM, Savos A, Kalil A, et al. CNS immune responses following experimental stroke. *Neurocrit Care.* (2010) 12:274–84. doi: 10.1007/s12028-009-9270-4
63. Zierath D, Schulze J, Kunze A, Drogomiretskiy O, Nhan D, Jaspers B, et al. The immunologic profile of adoptively transferred lymphocytes influences stroke outcome of recipients. *J Neuroimmunol.* (2013) 263:28–34. doi: 10.1016/j.jneuroim.2013.07.014
64. Ren X, Akiyoshi K, Grafe MR, Vandenbark AA, Hurn PD, Herson PS, et al. Myelin specific cells infiltrate MCAO lesions and exacerbate stroke severity. *Metab Brain Dis.* (2012) 27:7–15. doi: 10.1007/s11011-011-9267-5
65. Zierath D, Hadwin J, Savos A, Carter KT, Kunze A, Becker KJ. Anamnestic recall of stroke related deficits – an animal model. *Stroke.* (2010) 41:2653–60. doi: 10.1161/STROKEAHA.110.592865
66. Buttini M, Limonta S, Boddeke HW. Peripheral administration of lipopolysaccharide induces activation of microglial cells in rat brain. *Neurochem Int.* (1996) 29:25–35. doi: 10.1016/0197-0186(95)00141-7
67. Zierath D, Kunze A, Fecteau L, Becker K. Promiscuity of autoimmune responses to MBP after stroke. *J Neuroimmunol.* (2015) 285:101–5. doi: 10.1016/j.jneuroim.2015.05.024
68. Becker KJ, McCarron RM, Ruetzler C, Laban O, Sternberg E, Flanders KC, et al. Immunologic tolerance to myelin basic protein decreases stroke size after transient focal cerebral ischemia. *Proc Natl Acad Sci USA.* (1997) 94:10873–8. doi: 10.1073/pnas.94.20.10873
69. Becker K, Kindrick D, McCarron R, Hallenbeck J, Winn R. Adoptive transfer of myelin basic protein-tolerized splenocytes to naive animals reduces infarct size: a role for lymphocytes in ischemic brain injury? *Stroke.* (2003) 34:1809–15. doi: 10.1161/01.STR.0000078308.77727.EA
70. Subramanian S, Zhang B, Kosaka Y, Burrows GG, Grafe MR, Vandenbark AA, et al. Recombinant T cell receptor ligand treats experimental stroke. *Stroke.* (2009) 40:2539–45. doi: 10.1161/STROKEAHA.108.543991
71. Huan J, Subramanian S, Jones R, Rich C, Link J, Mooney J, et al. Monomeric recombinant TCR ligand reduces relapse rate and severity of experimental autoimmune encephalomyelitis in SJL/J mice through cytokine switch. *J Immunol.* (2004) 172:4556–66. doi: 10.4049/jimmunol.172.7.4556
72. Becker KJ, Kalil AJ, Tanzi P, Zierath DK, Savos AV, Gee JM, et al. Autoimmune responses to the brain after stroke are associated with worse outcome. *Stroke.* (2011) 42:2763–9. doi: 10.1161/STROKEAHA.111.619593
73. Becker K. Autoimmune responses to brain following stroke. *Transl Stroke Res.* (2012) 3:310–7. doi: 10.1007/s12975-012-0154-0
74. Klehmet J, Hoffmann S, Walter G, Meisel C, Meisel A. Stroke induces specific alteration of T memory compartment controlling auto-reactive CNS antigen-specific T cell responses. *J Neurol Sci.* (2016) 368:77–83. doi: 10.1016/j.jns.2016.06.039
75. Romer C, Engel O, Winek K, Hochmeister S, Zhang T, Royl G, et al. Blocking stroke-induced immunodeficiency increases CNS antigen-specific autoreactivity but does not worsen functional outcome after experimental stroke. *J Neurosci.* (2015) 35:7777–94. doi: 10.1523/JNEUROSCI.1532-14.2015
76. Chen Y, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science.* (1994) 265:1237–40. doi: 10.1126/science.7520605
77. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. *Blood.* (2008) 112:1570–80. doi: 10.1182/blood-2008-02-078071
78. Hoffman W, Lakkis FG, Chalasani G. B cells, antibodies, and more. *Clin J Am Soc Nephrol.* (2016) 11:137–54. doi: 10.2215/CJN.09430915
79. Levin EC, Acharya NK, Han M, Zavareh SB, Sedeyn JC, Venkataraman V, et al. Brain-reactive autoantibodies are nearly ubiquitous in human sera and may be linked to pathology in the context of blood-brain barrier breakdown. *Brain Res.* (2010) 1345:221–32. doi: 10.1016/j.brainres.2010.05.038
80. Hwang JK, Alt FW, Yeap LS. Related mechanisms of antibody somatic hypermutation and class switch recombination. *Microbiol Spectr.* (2015) 3:MDNA3-2014. doi: 10.1128/microbiolspec.MDNA3-0037-2014
81. Blum JS, Wearsch PA, Cresswell P. Pathways of antigen processing. *Annu Rev Immunol.* (2013) 31:443–73. doi: 10.1146/annurev-immunol-032712-095910
82. Liesz A, Dalpke A, Mracsko E, Antoine DJ, Roth S, Zhou W, et al. DAMP signaling is a key pathway inducing immune modulation after brain injury. *J Neurosci.* (2015) 35:583–98. doi: 10.1523/JNEUROSCI.2439-14.2015
83. Doyle KP, Buckwalter MS. Does B lymphocyte-mediated autoimmunity contribute to post-stroke dementia? *Brain Behav Immun.* (2017) 64:1–8. doi: 10.1016/j.bbi.2016.08.009
84. Shi K, Wood K, Shi FD, Wang X, Liu Q. Stroke-induced immunosuppression and poststroke infection. *Stroke Vasc Neurol.* (2018) 3:34–41. doi: 10.1136/svn-2017-000123
85. Rostrom B, Link B. Oligoclonal immunoglobulins in cerebrospinal fluid in acute cerebrovascular disease. *Neurology.* (1981) 31:590–6. doi: 10.1212/WNL.31.5.590
86. Tsementzis SA, Chao SW, Hitchcock ER, Gill JS, Beevers DG. Oligoclonal immunoglobulin G in acute subarachnoid hemorrhage and stroke. *Neurology.* (1986) 36:395–7. doi: 10.1212/WNL.36.3.395
87. Prüss H, Iggena D, Baldinger T, Prinz V, Meisel A, Endres M, et al. Evidence of intrathecal immunoglobulin synthesis in stroke: a cohort study. *Arch Neurol.* (2012) 69:714–7. doi: 10.1001/archneurol.2011.3252
88. Bornstein NM, Aronovich B, Korczyn AD, Shavit S, Michaelson DM, Chapman J. Antibodies to brain antigens following stroke. *Neurology.* (2001) 56:529–30. doi: 10.1212/WNL.56.4.529
89. Barry H, Byrne S, Barrett E, Murphy KC, Cotter DR. Anti-N-methyl-d-aspartate receptor encephalitis: review of clinical presentation, diagnosis and treatment. *BJPsych Bull.* (2015) 39:19–23. doi: 10.1192/pb.bp.113.045518
90. Dambinova SA, Khounteev GA, Izykenova GA, Zavolokov IG, Ilyukhina AY, Skoromets AA. Blood test detecting autoantibodies to N-methyl-D-aspartate neuroreceptors for evaluation of patients with transient ischemic attack and stroke. *Clin Chem.* (2003) 49:1752–62. doi: 10.1373/49.10.1752
91. Bokesch PM, Izykenova GA, Justice JB, Easley KA, Dambinova SA. NMDA receptor antibodies predict adverse neurological outcome after cardiac surgery in high-risk patients. *Stroke.* (2006) 37:1432–6. doi: 10.1161/01.STR.0000221295.14547.c8
92. Weissman JD, Khunteev GA, Heath R, Dambinova SA. NR2 antibodies: risk assessment of transient ischemic attack (TIA)/stroke in patients with history of isolated and multiple cerebrovascular events. *J Neurol Sci.* (2011) 300:97–102. doi: 10.1016/j.jns.2010.09.023
93. Kalev-Zylinska ML, Symes W, Little KCE, Sun P, Wen D, Qiao L, et al. Stroke patients develop antibodies that react with components of nmda receptor subunit 1 in proportion to lesion size. *Stroke.* (2013) 44:2212–9. doi: 10.1161/STROKEAHA.113.001235
94. Hammer C, Stepniak B, Schneider A, Papiol S, Tantra M, Begemann M, et al. Neuropsychiatric disease relevance of circulating anti-NMDA receptor autoantibodies depends on blood-brain barrier integrity. *Mol Psychiatry.* (2014) 19:1143–9. doi: 10.1038/mp.2013.110
95. Elvington A, Atkinson C, Kulik L, Zhu H, Yu J, Kindy MS, et al. Pathogenic natural antibodies propagate cerebral injury following ischemic stroke in mice. *J Immunol.* (2012) 188:1460–8. doi: 10.4049/jimmunol.1102132
96. Chen Y, Bodhankar S, Murphy SJ, Vandenbark AA, Alkayed NJ, Offner H. Intrastriatal B-cell administration limits infarct size after stroke in B-cell deficient mice. *Metab Brain Dis.* (2012) 27:487–93. doi: 10.1007/s11011-012-9317-7
97. Ren X, Akiyoshi K, Dziennis S, Vandenbark AA, Herson PS, Hurn PD, et al. Regulatory B cells limit CNS inflammation and neurologic deficits in murine experimental stroke. *J Neurosci.* (2011) 31:8556–63. doi: 10.1523/JNEUROSCI.1623-11.2011
98. Bodhankar S, Chen Y, Vandenbark AA, Murphy SJ, Offner H. IL-10-producing B-cells limit CNS inflammation and infarct volume in experimental stroke. *Metab Brain Dis.* (2013) 28:375–86. doi: 10.1007/s11011-013-9413-3

99. Becker KJ, Tanzi P, Zierath D, Buckwalter MS. Antibodies to myelin basic protein are associated with cognitive decline after stroke. *J Neuroimmunol.* (2016) 295–296:9–11. doi: 10.1016/j.jneuroim.2016.04.001
100. Matsushita T, Yanaba K, Bouaziz JD, Fujimoto M, Tedder TF. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *J Clin Invest.* (2008) 118:3420–30. doi: 10.1172/JCI 36030
101. Ankeny DP, Lucin KM, Sanders VM, McGaughy VM, Popovich PG. Spinal cord injury triggers systemic autoimmunity: evidence for chronic B lymphocyte activation and lupus-like autoantibody synthesis. *J Neurochem.* (2006) 99:1073–87. doi: 10.1111/j.1471-4159.2006.04147.x
102. Ankeny DP, Guan Z, Popovich PG. B cells produce pathogenic antibodies and impair recovery after spinal cord injury in mice. *J Clin Invest.* (2009) 119:2990–9. doi: 10.1172/JCI39780
103. Yeoh S, Bell ED, Monson KL. Distribution of blood-brain barrier disruption in primary blast injury. *Ann Biomed Eng.* (2013) 41:2206–14. doi: 10.1007/s10439-013-0805-7
104. Daneshvar DH, Goldstein LE, Kiernan PT, Stein TD, McKee AC. Post-traumatic neurodegeneration and chronic traumatic encephalopathy. *Mol Cell Neurosci.* (2015) 66:81–90. doi: 10.1016/j.mcn.2015.03.007
105. Simon DW, McGeachy MJ, Bayir H, Clark RSB, Loane DJ, Kochanek PM. The far-reaching scope of neuroinflammation after traumatic brain injury. *Nat Rev Neurol.* (2017) 13:171–91. doi: 10.1038/nrneurol.2017.13
106. Dorsett CR, McGuire JL, DePasquale EA, Gardner AE, Floyd CL, McCullumsmith RE. Glutamate neurotransmission in rodent models of traumatic brain injury. *J Neurotrauma.* (2017) 34:263–72. doi: 10.1089/neu.2015.4373
107. Anthonymuthu TS, Kenny EM, and Bayir H. Therapies targeting lipid peroxidation in traumatic brain injury. *Brain Res.* (2016) 1640:57–76. doi: 10.1016/j.brainres.2016.02.006
108. Jassam YN, Izzy S, Whalen M, McGavern DB, El Khoury J. Neuroimmunology of traumatic brain injury: time for a paradigm shift. *Neuron.* (2017) 95:1246–65. doi: 10.1016/j.neuron.2017.07.010
109. Siman R, Toraskar N, Dang A, McNeil E, McGarvey M, Plaum J, et al. A panel of neuron-enriched proteins as markers for traumatic brain injury in humans. *J Neurotrauma.* (2009) 26:1867–77. doi: 10.1089/neu.2009.0882
110. Mondello S, Robicsek SA, Gabrielli A, Brophy GM, Papa L, Tepas J, et al. αII-spectrin breakdown products (SBDPs): diagnosis and outcome in severe traumatic brain injury patients. *J Neurotrauma.* (2010) 27:1203–13. doi: 10.1089/neu.2010.1278
111. Hajduková L, Sobek O, Prchalová D, Bílková Z, Koudelková M, Lukášková J, et al. Biomarkers of brain damage: S100B and NSE concentrations in cerebrospinal fluid–a normative study. *BioMed Res Int.* (2015) 2015: 379071. doi: 10.1155/2015/379071
112. Mondello S, Kobeissy F, Vestri A, Hayes RL, Kochanek PM, Berger RP. Serum concentrations of ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein after pediatric traumatic brain injury. *Sci Rep.* (2016) 6:28203. doi: 10.1038/srep28203
113. Papa L, Brophy GM, Welch RD, Lewis LM, Braga CF, Tan CN, et al. Time course and diagnostic accuracy of glial and neuronal blood biomarkers gfap and uch-l1 in a large cohort of trauma patients with and without mild traumatic brain injury. *JAMA Neurol.* (2016) 73:551–60. doi: 10.1001/jamaneurol.2016.0039
114. Takala RS, Posti JP, Runtti H, Newcombe VF, Outtrim J, Katila AJ, et al. Glial fibrillary acidic protein and ubiquitin C-terminal hydrolase-L1 as outcome predictors in traumatic brain injury. *World Neurosurg.* (2016) 87:8–20. doi: 10.1016/j.wneu.2015.10.066
115. Thelin EP, Jeppsson E, Frostell A, Svensson M, Mondello S, Bellander B-M, et al. Utility of neuron-specific enolase in traumatic brain injury; relations to S100B levels, outcome, and extracranial injury severity. *Crit Care.* (2016) 20:285. doi: 10.1186/s13054-016-1450-y
116. Tanriverdi F, De Bellis A, Bizzarro A, Sinisi AA, Bellastella G, Pane E, et al. Antipituitary antibodies after traumatic brain injury: is head trauma-induced pituitary dysfunction associated with autoimmunity? *Eur J Endocrinol.* (2008) 159:7–13. doi: 10.1530/EJE-08-0050
117. Tanriverdi F, De Bellis A, Battaglia M, Bellastella G, Bizzarro A, Sinisi AA, et al. Investigation of antihypothalamus and antipituitary antibodies in amateur boxers: is chronic repetitive head trauma-induced pituitary dysfunction associated with autoimmunity? *Eur J Endocrinol.* (2010) 162:861–7. doi: 10.1530/EJE-09-1024
118. Tanriverdi F, De Bellis A, Ulutabanca H, Bizzarro A, Sinisi AA, Bellastella G, et al. A five year prospective investigation of anterior pituitary function after traumatic brain injury: is hypopituitarism long-term after head trauma associated with autoimmunity? *J Neurotrauma.* (2013) 30:1426–33. doi: 10.1089/neu.2012.2752
119. Goyal A, Failla MD, Niyonkuru C, Amin K, Fabio A, Berger RP, et al. S100b as a prognostic biomarker in outcome prediction for patients with severe traumatic brain injury. *J Neurotrauma.* (2013) 30:946–57. doi: 10.1089/neu.2012.2579
120. Thelin EP, Nelson DW, Bellander B-M. A review of the clinical utility of serum S100B protein levels in the assessment of traumatic brain injury. *Acta Neurochirurg.* (2017) 159:209–25. doi: 10.1007/s00701-016-3046-3
121. Marchi N, Bazarian JJ, Puvenna V, Janigro M, Ghosh C, Zhong J, et al. Consequences of repeated blood-brain barrier disruption in football players. *PLoS ONE.* (2013) 8:e56805. doi: 10.1371/journal.pone.0056805
122. Ngankam L, Kazantseva NV, Gerasimova MM. [Immunological markers of severity and outcome of traumatic brain injury]. *Zh Nevrol Psikhiatr Im S S Korsakova.* (2011) 111:61–5.
123. Zhang Z, Zoltewicz JS, Mondello S, Newsom KJ, Yang Z, Yang B, et al. Human traumatic brain injury induces autoantibody response against glial fibrillary acidic protein and its breakdown products. *PLoS ONE.* (2014) 9:e92698. doi: 10.1371/journal.pone.0092698
124. Wang KK, Yang Z, Yue JK, Zhang Z, Winkler EA, Puccio AM, et al. Plasma anti-glial fibrillary acidic protein autoantibody levels during the acute and chronic phases of traumatic brain injury: a transforming research and clinical knowledge in traumatic brain injury pilot study. *J Neurotrauma.* (2016) 33:1270–7. doi: 10.1089/neu.2015.3881
125. Goryunova AV, Bazarnaya NA, Sorokina EG, Semenova NY, Globa OV, Semenova ZhB, et al. Glutamate receptor autoantibody concentrations in children with chronic post-traumatic headache. *Neurosci Behav Physiol.* (2007) 37:761–4. doi: 10.1007/s11055-007-0079-3
126. Shively S, Scher AI, Perl DP, Diaz-Arrastia R. Dementia resulting from traumatic brain injury: what is the pathology? *Arch Neurol.* (2012) 69:1245–51. doi: 10.1001/archneurol.2011.3747
127. Montgomery S, Hiyoshi A, Burkill S, Alfredsson L, Bahmanyar S, Olsson T. Concussion in adolescence and risk of multiple sclerosis. *Ann Neurol.* (2017) 82:554–61. doi: 10.1002/ana.25036
128. Swanberg M, Lidman O, Padyukov L, Eriksson P, Akesson E, Jagodic M, et al. MHC2TA is associated with differential MHC molecule expression and susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction. *Nat Genet.* (2005) 37:486–94. doi: 10.1038/ng1544
129. Johnson VE, Stewart JE, Begbie FD, Trojanowski JQ, Smith DH, Stewart W. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. *Brain.* (2013) 136:28–42. doi: 10.1093/brain/aws322
130. Ramlackhansingh AF, Brooks DJ, Greenwood RJ, Bose SK, Turkheimer FE, Kinnunen KM, et al. Inflammation after trauma: microglial activation and traumatic brain injury. *Ann Neurol.* (2011) 70:374–83. doi: 10.1002/ana.22455
131. Clausen F, Lorant T, Lewen A, Hillered L. T lymphocyte trafficking: a novel target for neuroprotection in traumatic brain injury. *J Neurotrauma.* (2007) 24:1295–307. doi: 10.1089/neu.2006.0258
132. Czigner A, Mihaly A, Farkas O, Buki A, Krisztin-Peva B, Dobo E, et al. Kinetics of the cellular immune response following closed head injury. *Acta Neurochir.* (2007) 149:281–9. doi: 10.1007/s00701-006-1095-8
133. Ling C, Sandor M, Suresh M, Fabry Z. Traumatic injury and the presence of antigen differentially contribute to T-cell recruitment in the CNS. *J Neurosci.* (2006) 26:731–41. doi: 10.1523/JNEUROSCI.3502-05.2006
134. Weckbach S, Neher M, Losacco JT, Bolden AL, Kulik L, Flierl MA, et al. Challenging the role of adaptive immunity in neurotrauma: Rag1(-/-) mice lacking mature B and T cells do not show neuroprotection after closed head injury. *J Neurotrauma.* (2012) 29:1233–42. doi: 10.1089/neu.2011.2169
135. Mencl S, Hennig N, Hopp S, Schuhmann MK, Albert-Weissenberger C, Sirén AL, et al. FTY720 does not protect from traumatic brain injury in mice despite reducing posttraumatic inflammation. *J Neuroimmunol.* (2014) 274:125–31. doi: 10.1016/j.jneuroim.2014.07.010

136. Cox AL, Coles AJ, Nortje J, Bradley PG, Chatfield DA, Thompson SJ, et al. An investigation of auto-reactivity after head injury. *J Neuroimmunol.* (2006) 174:180–6. doi: 10.1016/j.jneuroim.2006.01.007
137. Hirschberg DL, Moalem G, He J, Mor F, Cohen IR, Schwartz M. Accumulation of passively transferred primed T cells independently of their antigen specificity following central nervous system trauma. *J Neuroimmunol.* (1998) 89:88–96. doi: 10.1016/S0165-5728(98)00118-0
138. Moalem G, Leibowitz–Amit R, Yoles E, Mor F, Cohen IR, Schwartz M. Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. *Nat Med.* (1999) 5:49. doi: 10.1038/4734
139. Fisher J, Levkovitch-Verbin H, Schori H, Yoles E, Butovsky O, Kaye JF, et al. Vaccination for neuroprotection in the mouse optic nerve: implications for optic neuropathies. *J Neurosci.* (2001) 21:136–42. doi: 10.1523/JNEUROSCI.21-01-00136.2001
140. Kipnis J, Mizrahi T, Hauben E, Shaked I, Shevach E, Schwartz M. Neuroprotective autoimmunity: naturally occurring CD4+CD25+ regulatory T cells suppress the ability to withstand injury to the central nervous system. *Proc Natl Acad Sci USA.* (2002) 99:15620–5. doi: 10.1073/pnas.232565399
141. Kil K, Zang YC, Yang D, Markowski J, Fuoco GS, Vendetti GC, et al. T cell responses to myelin basic protein in patients with spinal cord injury and multiple sclerosis. *J Neuroimmunol.* (1999) 98:201–7. doi: 10.1016/S0165-5728(99)00057-0
142. Jones TB, Hart RP, Popovich PG. Molecular control of physiological and pathological T-cell recruitment after mouse spinal cord injury. *J Neurosci.* (2005) 25:6576–83. doi: 10.1523/JNEUROSCI.0305-05.2005
143. Ibarra A, Jiménez A, Cortes C, Correa D. Influence of the intensity, level and phase of spinal cord injury on the proliferation of T cells and T-cell-dependent antibody reactions in rats. *Spinal Cord.* (2006) 45:380. doi: 10.1038/sj.sc.3101972
144. Zajarías-Fainsod D, Carrillo-Ruiz J, Mestre H, Grijalva I, Madrazo I, Ibarra A. Autoreactivity against myelin basic protein in patients with chronic paraplegia. *Eur Spine J.* (2012) 21:964–70. doi: 10.1007/s00586-011-2060-7
145. Davies AL, Hayes KC, Dekaban GA. Clinical correlates of elevated serum concentrations of cytokines and autoantibodies in patients with spinal cord injury. *Arch Phys Med Rehabil.* (2007) 88:1384–93. doi: 10.1016/j.apmr.2007.08.004
146. Olsson T, Diener P, Ljungdahl A, Hojeberg B, van der Meide PH, Kristensson K. Facial nerve transection causes expansion of myelin autoreactive T cells in regional lymph nodes and T cell homing to the facial nucleus. *Autoimmunity.* (1992) 13:117–26. doi: 10.3109/08916939209001912
147. Hauben E, Butovsky O, Nevo U, Yoles E, Moalem G, Agranov E, et al. Passive or active immunization with myelin basic protein promotes recovery from spinal cord contusion. *J Neurosci.* (2000) 20:6421. doi: 10.1523/JNEUROSCI.20-17-06421.2000
148. Jones TB, Basso DM, Sodhi A, Pan JZ, Hart RP, MacCallum RC, et al. Pathological CNS autoimmune disease triggered by traumatic spinal cord injury: implications for autoimmune vaccine therapy. *J Neurosci.* (2002) 22:2690–700. doi: 10.1523/JNEUROSCI.22-07-02690.2002
149. Muhallab S, Lundberg C, Gielen AW, Lidman O, Svenningsson A, Piehl F, et al. Differential expression of neurotrophic factors and inflammatory cytokines by myelin basic protein-specific and other recruited T cells infiltrating the central nervous system during experimental autoimmune encephalomyelitis. *Scand J Immunol.* (2002) 55:264–73. doi: 10.1046/j.0300-9475.2002.01038.x
150. Olsson T, Lidman O, Piehl F. Harm or heal–divergent effects of autoimmune neuroinflammation? *Trends Immunol.* (2003) 24:5–6; author reply 7–8. doi: 10.1016/S1471-4906(02)00003-0
151. Walsh JT, Hendrix S, Boato F, Smirnov I, Zheng J, Lukens JR, et al. MHCII-independent CD4+ T cells protect injured CNS neurons via IL-4. *J Clin Invest.* (2015) 125:699–714. doi: 10.1172/JCI76210
152. Arevalo-Martin A, Grassner L, Garcia-Ovejero D, Paniagua-Torija B, Barroso-Garcia G, Arandilla AG, et al. Elevated autoantibodies in subacute human spinal cord injury are naturally occurring antibodies. *Front Immunol.* (2018) 9:2365. doi: 10.3389/fimmu.2018.02365
153. Saltzman JW, Battaglino RA, Salles L, Jha P, Sudhakar S, Garshick E, et al. B-cell maturation antigen, a proliferation-inducing ligand, and B-cell activating factor are candidate mediators of spinal cord injury-induced autoimmunity. *J Neurotrauma.* (2013) 30:434–40. doi: 10.1089/neu.2012.2501

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BRIEF RESEARCH REPORT
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Clinical Features and Inflammatory Markers in Autoimmune Encephalitis Associated With Antibodies Against Neuronal Surface in Brazilian Patients

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Acute encephalitis is a debilitating neurological disorder associated with brain inflammation and rapidly progressive encephalopathy. Autoimmune encephalitis (AE) is increasingly recognized as one of the most frequent causes of encephalitis, however signs of inflammation are not always present at the onset which may delay the diagnosis. We retrospectively assessed patients with AE associated with antibodies against neuronal surface diagnosed in reference centers in Northeast of Brazil between 2014 to 2017. CNS inflammatory markers were defined as altered CSF (pleocytosis >5 cells/mm^3) and/or any brain parenchymal MRI signal abnormality. Thirteen patients were evaluated, anti-NMDAR was the most common antibody found (10/13, 77%), followed by anti-LGI1 (2/13, 15%), and anti-AMPAR (1/13, 7%). Median time to diagnosis was 4 months (range 2–9 months). Among these 13 patients, 6 (46.1%) had inflammatory markers and when compared to those who did not present signs of inflammation, there were no significant differences regarding the age of onset, time to diagnosis and modified Rankin scale score at the last visit. Most of the patients presented partial or complete response to immunotherapy during follow-up. Our findings suggest that the presence of inflammatory markers may not correlate with clinical presentation or prognosis in patients with AE associated with antibodies against neuronal surface. Neurologists should be aware to recognize clinical features of AE and promptly request antibody testing even without evidence of inflammation in CSF or MRI studies.

Keywords: autoimmune encephalitis, Inflammatory biomarkers, neuronal surface antibody, NMDAR, LGI1, AMPAR, low-income population

INTRODUCTION

Autoimmune encephalitis (AE) is increasingly recognized as one of the most frequent causes of encephalitis (1, 2). The identification of antibodies against neuronal surface antigens as biomarkers of treatable neurological syndromes has changed the approach to encephalitis and other inflammatory central nervous system (CNS) disorders (1). The differential diagnosis of AE may be complicated because signs of inflammation on neuroimaging or cerebrospinal fluid (CSF) studies may be absent, especially in patients over 60 years of age (3). This raises the question of whether these findings would be replicated in consecutive, unselected patients without age restriction. In Brazil there is a small series of three patients reported with anti-NMDAR antibodies in the city of Brasília (4), and a study in rapidly progressive dementia in which 10 cases of anti-NMDAR encephalitis were reported in São Paulo (5). The aim of this study was to characterize clinical features and outcome in a cohort of consecutive patients with AE from a single city in Northeast Brazil and to compare patients that presented or not findings suggestive of active inflammation.

MATERIALS AND METHODS

Patients and Clinical Data

We retrospectively identified consecutive patients whose serum or CSF samples tested positive for neuronal antibodies in the city of Fortaleza, state of Ceará, northeast Brazil, from January 2014 to March 2017. This study was conducted in the main neurological comprehensive care centers of our city (Hospital Geral de Fortaleza, Walter Cantidio University Hospital and Albert Sabin Children's Hospital), covering a population of almost 2.7 million. Thus, we believe that most patients diagnosed with AE during this period were included in our cohort.

Blood and CSF samples analyses were performed at IDIBAPS, Barcelona, with support from Dr. Josep Dalmau. All samples were screened for reactivity using rat brain sections and then through cell-based assay with HEK293 recombinantly expressing N-methyl-D-aspartate receptor (NMDAR), leucine-rich glioma inactivated-1 (LGI1), contactin-associated protein-like 2 (CASPR2), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), gamma-aminobutyric acid (GABA)b receptor or GABAa receptor as previously reported (6).

AE was defined by the presence of all three of the following criteria: (1) subacute onset (rapid progression <3 months) of working memory deficits (short-term memory loss), altered mental status, or psychiatric symptoms; (2) at least one of the following: new focal CNS findings, seizures not explained by a previously known seizure disorder, CSF pleocytosis (white blood cell count > 5 cells/mm^3), or magnetic resonance imaging (MRI) features suggestive of encephalitis; and (3) reasonable exclusion of alternative causes (1). Exclusion criteria included syndromes predominantly involving the spinal cord or peripheral nerve.

Electroencephalogram (EEG) monitoring was performed in all patients. The following EEG variables were collected: presence or absence of electrographic seizures; clinical or subclinical seizures; diffuse slowing; focal slowing; rhythmic delta activity; excessive beta activity; and presence of extreme delta brush. MRI included T1-weighted, T2-weighted, fluid attenuated inversion recovery (FLAIR), diffusion weighted imaging (DWI) and gradient echo sequences. CNS inflammatory markers were defined as altered CSF (pleocytosis > 5 cells/mm^3) and/or any brain MRI abnormality suggestive of encephalitis (mesial temporal T2 hypersignal or lesions suggestive of demyelination).

The modified Rankin Scale (mRS) was used to assess disability at onset, at the worst clinical status and at the last visit. A good outcome was defined as mRS score of 0–2 at the last follow-up and a poor outcome as mRS score > 2. Tumors were screened for at the initial presentation and every 6 months with thoracic and abdominal computed tomography and vaginal ultrasound or ovarian MRI for ovarian teratomas.

Statistical Analyses

Categorical variables were described as absolute frequencies and percentages, while numerical variables were described as medians and ranges. Comparative analyses between patients with and without inflammatory changes were performed using the Mann-Whitney U test or 2-sample t-test as appropriate for continuous variables, and Fisher's exact test for categorical variables. Two-tailed p-values of < 0.05 were considered statistically significant. Statistical analyses were performed IBM-SPSS version 18.0 (IBM, Armonk, NY, USA).

Ethical Approval

All patients gave their written consent for the storage and use of clinical samples for research purposes and to be included in this report. The study was approved by the local ethics committee (number of approval: 2.652.778).

RESULTS

Demographic and Clinical Features

Among 13 patients identified, 9 (69%) were female and median age was 17 years (range 4–75 years). Anti-NMDAR was the most common antibody found (10/13, 77%), followed by anti-LGI1 (2/13, 15%), and anti-AMPAR (1/13, 7%). Median time to diagnosis was 4 months (range 2–9 months) Before an AE was considered, patients received other diagnosis including herpes simplex encephalitis, primary psychosis, and neuroleptic malignant syndrome.

The most common initial presentation was encephalopathy with memory loss and behavioral changes suggestive of AE seen in 11/13 (84%): all 10 patients with anti-NMDAR and 1 patient with anti-AMPAR. Psychiatric symptoms presented before neurologic dysfunction in 4/13 patients. Dyskinesias, typically orofacial, were present in 9/13 patients and hand dyskinesias in 5/13. Dystonia was seen in 3/13 patients. Faciobrachial dystonic seizures (FBDS) followed by encephalopathy and memory loss were present in both anti-LGI1 patients. Only one patient with anti-NMDAR presented with refractory status epilepticus followed by orofacial and hand dyskinesias, dystonia and encephalopathy after resolution. Hyponatremia was present in three patients, one with anti-AMPAR and in both with anti-LGI1, and improved after immunotherapy. Regarding associated

tumors, there was only one case of small cell lung carcinoma in the patient with anti-AMPAR. **Table 1** presents clinical features of patients with AE.

Treatment and Outcomes

Treatment consisted in intravenous methylprednisolone alone (3/13, 23%), methylprednisolone with immunoglobulin (9/13, 69%), or immunoglobulin alone (1/13, 7%). Most patients (8/13, 61%) responded to the first-line therapy. In three patients with no significant improvement after 2 weeks, a second-line therapy was done with rituximab, and in one patient combined with cyclophosphamide. Patients were severely impaired at initial presentation, with a median mRS score of 5 (range 4–5). Most showed significant improvement after treatment, where 9/13 (69%) achieved a good outcome (mRS $\leq$ 2). Median follow up duration was 22 months (range 3–48). Three patients had a mRS $\geq$ 3 at the last visit, including one patient with anti-NMDAR who died of central line associated sepsis while receiving parenteral nutrition for a drug-induced pancreatitis (immunoglobulin had been administered 2 weeks earlier with neurological improvement). Maintenance treatment was conducted at the decision of attending physicians. Most patients received oral steroids for a variable period (median of 5 months). None received chronic immunosuppression. There were no relapses during follow-up.

CNS Inflammatory Markers and EEG Findings

Most patients had normal MRI (10/13, 77%). From those with MRI abnormalities (3/13, 23%): two (both with anti-NMDAR) had extralimbic findings with nonspecific cortical hyperintensities; and one had bilateral hippocampal hyperintensities compatible with limbic encephalitis and left basal ganglia hypersignal (a case of anti-LGI1 with unilateral FBDS). Follow up MRI scans were performed in two patients. In one of the anti-LGI1 patients atrophy of right caudate and putamen was observed after 1 year. In an anti-NMDAR patient complete resolution of subcortical abnormalities and no atrophy were observed after 8 months. CSF pleocytosis was seen in less than a half of patients (5/13, 38.4%). None of the patients had positive oligoclonal bands. All 13 patients showed EEG abnormalities; they were mostly nonspecific slowing of baseline activity (10/13, 77%). Two patients, both with anti-NMDAR, showed an "extreme delta brush" pattern. One patient with anti-NMDAR presented with status epilepticus. In both patients with FBDS, there was no EEG correlation with abnormal movements.

Seven patients (53.8%) had no signs of inflammation on CSF or MRI. No evidence of inflammation was seen in half of patients with anti-NMDAR, in one patient with anti-LGI1 and in the only patient with anti-AMPAR. A good outcome at the last visit was seen in 83.3% of the patients with inflammatory changes and in 57.1% of those without these changes ($p = 0.559$). No significant differences in age of onset, response to immunotherapy, time to diagnosis and prognosis were observed between patients with or without inflammatory markers. **Table 2** shows a comparison of clinical features between patients with AE with and without inflammatory changes.

DISCUSSION

To the best of our knowledge, this is the largest cohort of patients with AE reported in Brazil. The estimated incidence rate was 0.16/100,000 person-years, five times lower than the incidence of 0.8/100,000 found in a study in Minesotta (7). We found a high frequency of normal MRI and CSF studies and our findings suggest no correlation between inflammatory markers and clinical presentation or prognosis. Some studies have found evidence of association of CSF changes with a worse outcome (8, 9) and MRI changes, particularly cerebellar atrophy in follow-up MRI (10), was negatively correlated with outcome. However, a recent systematic review suggested that early CSF and MRI abnormalities did not demonstrate a strong relationship with patient outcomes (11).

Major diagnostic criteria of encephalitis (of any cause) include the presentation of decreased level of consciousness, neuroimaging findings suggestive of inflammation, and CSF pleocytosis (12). Yet, a recent study applied the newly proposed AE diagnostic criteria to patients over 60 years of age. The authors confirmed these criteria are appropriate to identify patients with possible AE in the absence of evidence of CNS inflammation (3).

The most common antibody found in our study was anti-NMDAR, as reported in other series of AE patients (13). A recent study of 29 cases of anti-NMDAR encephalitis reported an absence of inflammatory markers in CSF in almost 50% of patients, and a delayed time to diagnosis in these cases (14). In our study, time to diagnosis was similar in anti-NMDAR encephalitis patients regardless of their inflammatory status.

NMDAR antibodies bind to an extracellular conformational epitope region close to the amino acid 369 of the GluN1 NMDAR subunit and reduce the receptor density leading to a reversible direct neuronal dysfunction (6, 15). Other autoantibodies may act through different mechanisms. GABAb receptor antibodies relocate the receptor to extrasynaptic sites; AMPAR antibodies reduce the receptor density at synaptic and extrasynaptic sites along with a reduction in miniature excitatory postsynaptic potentials; and LGI1-antibodies block the binding of LGI1 to ADAM22, resulting in a decrease of AMPAR (15–18). The pathogenesis of AE is probably more related to direct neuronal dysfunction caused by these antibodies than to inflammatory infiltrates or blood-brain barrier abnormalities (19). This could possibly explain why there are no signs of CNS inflammation on CSF or MRI in many cases. In both LGI1- and CASPR2-associated CNS syndromes, these antibodies are of IgG4 subclass, which do not fix complement, and the CSF is normal in most patients (20, 21).

Possible triggers for autoimmune response are under investigation. Ectopic expression of NMDAR in ovarian teratomas, for instance, is thought to trigger autoimmune response in paraneoplastic anti-NMDAR encephalitis (22). Recent literature strengthen the hypothesis of an infectious trigger based on the presence of a viral prodrome in the majority of non-paraneoplastic anti-NMDAR encephalitis, the development of NMDAR antibodies in 20% of patients after herpes simplex encephalitis and detection of past non-encephalitic herpes simplex virus 1 (HSV-1) infection in nearly

TABLE 1 | Summary of clinical presentations, paraclinical investigations and follow-up data of patients with autoimmune encephalopathy.

Case number	Age	Antibody	Clinical presentation	CSF	MRI	EEG	Time to diagnosis (months)	mRS (diagnosis)	mRS (last visit)
1	16	NMDA	Psychosis, seizures, encephalopathy, rigidity, dyskinesias, dysautonomia	Cells-2, Protein-57	Occipital subcortical hyperintensities	Extreme delta brush	4	5	0
2	16	NMDA	Seizures, encephalopathy, dyskinesias, dysautonomia, akinetic mutism	NL	NL	Extreme delta brush	4	5	0
3	17	NMDA	Psychosis, seizures, encephalopathy, myoclonus, dyskinesias, dysautonomia	NL	NL	Slow theta and delta waves	4	5	0
4	26	NMDA	Encephalopathy, dyskinesias, dysautonomia, dystonia	Cells-336, Protein-16	Cortical hyperintensities	Diffuse slowing	2	4	2
5	26	NMDA	Psychosis, myoclonus, encephalopathy, dyskinesias, seizures, dyasutonomia	Cells-22, Protein-20	NL	Diffuse slowing	4	4	0
6	4	NMDA	Seizures, agressiveness, encephalopathy, dyskinesias, akinetic mutism	NL	NL	Slow activity	3	5	4
7	5	NMDA	Encephalopathy, dyskinesias, dystonia	NL	NL	Diffuse slowing	4	4	0
8	14	NMDA	Status epilepticus, Encephalopathy, dyskinesias, dystonia	NL	NL	Status Epilepticus	5	5	6
9	23	NMDA	Psychosis, akinetic mutism, dyskinesias, dysautonomia	Cells−197, Protein-21	NL	Diffuse slowing	3	5	0
10	75	LGI1	Faciobrachial dystonic seizures, encephalopathy, memory loss, hyponatremia	Cells−10, Protein-40	Right basal nuclei and hipocampal	Diffuse slowing	6	5	3
11	73	LGI1	Faciobrachial dystonic seizures, encephalopathy, memory loss, hyponatremia	NL	NL	Diffuse slowing	9	4	0
12	67	AMPA	Hyponatremia, agitation, insomnia, memory loss	NL	NL	Diffuse slowing	3	4	3
13	17	NMDA	Seizures, agressiveness, mutism, memory loss, encephalopathy	Cells-16, Protein-29	NL	Diffuse slowing	5	5	1

NMDA, N-methyl-D-asparthate; LGI1, Leucine-rich-glioma-inactivated-1; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NL, Normal; CSF, Cerebrospinal fluid; MRI, Magnetic resonance image; mRS, modified Rankin Scale.

TABLE 2 | Comparison between patients with autoimmune encephalitis with and without markers of inflammation in brain MRI and CSF.

Variables	Without inflammation ($n = 7$)	With inflammation ($n = 6$)	*P*
Age, median (range)	16 (4–73)	24.5 (16–75)	0.197
Female, *n* (%)	85.7%	50%	0.265
Diagnostic delay, median, months	4.0	3.5	0.371
Clincal Syndrome At Presentation			
Possible encephalitis[a]	6 (85.7%)	5 (83.3%)	0.906
FBDS	1 (14.3%)	1 (16.7%)	0.906
Hyponatremia, *n* (%)	2 (28.6%)	1 (16.7%)	0.612
mRS at last visit, median	0	0.5	0.641
Status At Last Visit			
mRS 0–2, *n* (%)	4 (57.1%)	5 (83.3%)	0.559
mRS 3-6, *n* (%)	3 (28.6%)	1 (16.7%)	

FBDS, faciobrachial dystonic seizures; mRS, modified Rankin score. [a]According to the criteria of Graus et al. (1).

half of the patients with anti-NMDAR encephalitis vs. less than a quarter of the controls (23, 24). Studies in animal models also demonstrate that exposure to HSV-1 can elicit production of NMDAR antibodies as well as reduction of expression of these receptors in the hippocampus of mice exposed to serum of patients with anti-NMDAR encephalitis (25). Some cases of anti-NMDAR encephalitis following mycoplasma, Epstein–Barr, varicella zoster, or influenza infections have also been reported (26–28). Molecular mimicry and dysregulation of immunoregulatory pathways are some of the mechanisms proposed for the link between infections and induction of CNS autoimmunity (16). It is possible that other infectious agents, including zika and chikungunya viruses, which had high incidence rates in Northeast Brazil during the period of this study, might also act as activators of CNS immune response.

Concerning anti-LGI1 encephalitis, we observed a good response to initial treatment in the patient with normal MRI, while the one with T2 hyperintensities in putamen and caudate had a poorer response to first-line therapy with only slight improvement of FBDS and required additional therapy with rituximab. It has been suggested that these signal abnormalities are more common in the presence of FBDS and are related to time since disease onset, with T2 hypersignal disappearing with disease progression (29). Whether this could be related to disease severity is still not clear (30, 31).

In our cohort, only two patients had follow-up imaging for analysis. In both cases, associated with anti-LGI1 and anti-NMDAR, no hippocampal atrophy was observed as previously reported (32). In the follow-up image of the anti-LGI1 encephalitis, caudate and putamen atrophy was observed, and this patient presented a higher frequency and duration of FBDS. This has also been noticed in other series and raises the question about the nature of these events, suggesting that involvement of the basal nuclei causes the stereotyped movements and that they are ultimately a movement disorder rather than a seizure (32, 33).

The importance of testing new onset refractory status epilepticus (NORSE) patients for neuronal surface antibodies has been previously discussed, as many of these cases might have an autoimmune etiology (34). In our cohort, one patient with intellectual disability and stable epilepsy deteriorated and developed prolonged seizures progressing to status epilepticus, behavior changes, and hand dyskinesias (piano playing) and was then diagnosed as anti-NMDAR encephalitis. Testing patients with severe worsening of a previous epilepsy disorder without an obvious cause for deterioration may be warranted in some settings.

The lack of difference between patients with and without inflammatory changes in our study might be due to the small sample size, heterogeneity and lack of representativeness, since cases with only three antibodies types were included with most of the cases being anti-NMDAR. However, we believe this cohort is closer to a "real-world" scenario, as our centers are not specialized in autoimmune encephalopathies and we analyzed all patients who tested positive for neuronal antibodies.

The present study was the first to provide an estimate of incidence of AE in a developing country, where access to MRI and EEG is very limited and antibody testing is not available commercially. Our findings reinforce the importance of previously reported diagnostic clues like FBDS and a syndrome suggestive of anti-NMDAR encephalitis for diagnosis, as well as the sensitivity of Graus criteria for possible autoimmune encephalitis, allowing for early initiation of immunotherapy before antibody results are available. We also report on a good outcome despite many challenges to diagnosis and treatment in underdeveloped parts of the world.

ETHICS STATEMENT

Local Ethics Commitee: Hospital Universitário Walter Cantídio-Universidade Federal do Ceará.

We would like to outline that all patients provided written informed consent for the collection of samples and subsequent case report. All authors have read and agree with the manuscript publication. The local ethics committees approved the final version of this article.

AUTHOR CONTRIBUTIONS

PN and PB-N: conception and design of the work. PN, LM, FM, MK, CS, NM, MP, MS, and PB-N: acquisition, analysis, or interpretation of data for the work. PN, MP, MS, and PB-N: drafting the work. All authors were involved in critical revision of the manuscript for important intellectual content.

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REFERENCES

1. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol.* (2016) 15:391–404. doi: 10.1016/S1474-4422(15)00401-9
2. Dutra LA, Abrantes F, Toso FF, Pedroso JL, Barsottini OGP, Hoftberger R. Autoimmune encephalitis: a review of diagnosis and treatment. *Arq Neuropsiquiatr.* (2018) 76:41–9. doi: 10.1590/0004-282x20170176
3. Escudero D, Guasp M, Arino H, Gaig C, Martinez-Hernandez E, Dalmau J, et al. Antibody-associated CNS syndromes without signs of inflammation in the elderly. *Neurology.* (2017) 89:1471–5. doi: 10.1212/WNL.0000000000004541
4. De Maria PRS, Melo FOS, Aguiar MS, Gusmão RL, Oliveira PAL, Ferreira AGF, et al. Autoimmune encephalitis: clinical features and electroencephalographic patterns. Study of three cases and literatura review. *Rev Med Saude Brasilia.* (2017) 6:64–72.
5. Studart Neto A, Soares Neto HR, Simabukuro MM, Solla DJF, Goncalves MRR, Fortini I, et al. Rapidly progressive dementia: prevalence and causes in a neurologic unit of a tertiary hospital in Brazil. *Alzheimer Dis Assoc Disord.* (2017) 31:239–43. doi: 10.1097/WAD.0000000000000170
6. Gresa-Arribas N, Titulaer MJ, Torrents A, Aguilar E, McCracken L, Leypoldt F, et al. Antibody titres at diagnosis and during follow-up of anti-NMDA receptor encephalitis: a retrospective study. *Lancet Neurol.* (2014) 13:167–77. doi: 10.1016/S1474-4422(13)70282-5
7. Dubey D, Pittock SJ, Kelly CR, McKeon A, Lopez-Chiriboga AS, Lennon VA, et al. Autoimmune encephalitis epidemiology and a comparison to infectious encephalitis. *Ann Neurol.* (2018) 83:166–77. doi: 10.1002/ana.25131
8. de Montmollin E, Demeret S, Brule N, Conrad M, Dailler F, Lerolle N, et al. Anti-N-Methyl-d-Aspartate receptor encephalitis in adult patients requiring intensive care. *Am J Respir Crit Care Med.* (2017) 195:491–9. doi: 10.1164/rccm.201603-0507OC
9. Wang W, Li JM, Hu FY, Wang R, Hong Z, He L, et al. Anti-NMDA receptor encephalitis: clinical characteristics, predictors of outcome and the knowledge gap in southwest China. *Eur J Neurol.* (2016) 23:621–9. doi: 10.1111/ene.12911
10. Iizuka T, Kaneko J, Tominaga N, Someko H, Nakamura M, Ishima D, et al. Association of progressive cerebellar atrophy with long-term outcome in patients with anti-N-Methyl-d-Aspartate receptor encephalitis. *JAMA Neurol.* (2016) 73:706–13. doi: 10.1001/jamaneurol.2016.0232
11. Broadley J, Seneviratne U, Beech P, Buzzard K, Butzkueven H, O'Brien T, et al. Prognosticating autoimmune encephalitis: a systematic review. *J Autoimmun.* (2019) 96:24–34. doi: 10.1016/j.jaut.2018.10.014
12. Venkatesan A, Tunkel AR, Bloch KC, Lauring AS, Sejvar J, Bitnun A, et al. Case definitions, diagnostic algorithms, and priorities in encephalitis: consensus statement of the international encephalitis consortium. *Clin Infect Dis.* (2013) 57:1114–28. doi: 10.1093/cid/cit458
13. Granerod J, Ambrose HE, Davies NW, Clewley JP, Walsh AL, Morgan D, et al. Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. *Lancet Infect Dis.* (2010) 10:835–44. doi: 10.1016/S1473-3099(10)70222-X
14. Espinola-Nadurille M, Bautista-Gomez P, Flores J, Rivas-Alonso V, Perez-Esparza R, Solis-Vivanco R, et al. Non-inflammatory cerebrospinal fluid delays the diagnosis and start of immunotherapy in anti-NMDAR encephalitis. *Arq Neuropsiquiatr.* (2018) 76:2–5. doi: 10.1590/0004-282x20170179
15. Titulaer MJ, McCracken L, Gabilondo I, Armangue T, Glaser C, Iizuka T, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. *Lancet Neurol.* (2013) 12:157–65. doi: 10.1016/S1474-4422(12)70310-1
16. Hoftberger R. Neuroimmunology: an expanding frontier in autoimmunity. *Front Immunol.* (2015) 6:206. doi: 10.3389/fimmu.2015.00206
17. Leypoldt F, Armangue T, Dalmau J. Autoimmune encephalopathies. *Ann NY Acad Sci.* (2015) 1338:94–114. doi: 10.1111/nyas.12553
18. Ohkawa T, Fukata Y, Yamasaki M, Miyazaki T, Yokoi N, Takashima H, et al. Autoantibodies to epilepsy-related LGI1 in limbic encephalitis neutralize LGI1-ADAM22 interaction and reduce synaptic AMPA receptors. *J Neurosci.* (2013) 33:18161–74. doi: 10.1523/JNEUROSCI.3506-13.2013
19. Lancaster E, Dalmau J. Neuronal autoantigens–pathogenesis, associated disorders and antibody testing. *Nat Rev Neurol.* (2012) 8:380–90. doi: 10.1038/nrneurol.2012.99
20. Binks SNM, Klein CJ, Waters P, Pittock SJ, Irani SR. LGI1, CASPR2 and related antibodies: a molecular evolution of the phenotypes. *J Neurol Neurosurg Psychiatry.* (2018) 89:526–34. doi: 10.1136/jnnp-2017-315720
21. Yeo T, Chen Z, Yong KP, Wong PYW, Chai JYH, Tan K. Distinction between anti-VGKC-complex seropositive patients with and without anti-LGI1/CASPR2 antibodies. *J Neurol Sci.* (2018) 391:64–71. doi: 10.1016/j.jns.2018.05.020
22. Dalmau J, Gleichman AJ, Hughes EG, Rossi JE, Peng X, Lai M, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. *Lancet Neurol.* (2008) 7:1091–8. doi: 10.1016/S1474-4422(08)70224-2
23. Gable MS, Gavali S, Radner A, Tilley DH, Lee B, Dyner L, et al. Anti-NMDA receptor encephalitis: report of ten cases and comparison with viral encephalitis. *Eur J Clin Microbiol Infect Dis.* (2009) 28:1421–9. doi: 10.1007/s10096-009-0799-0
24. Salovin A, Glanzman J, Roslin K, Armangue T, Lynch DR, Panzer JA. Anti-NMDA receptor encephalitis and nonencephalitic HSV-1 infection. *Neurol Neuroimmunol Neuroinflamm.* (2018) 5:e458. doi: 10.1212/NXI.0000000000000458
25. Linnoila J, Pulli B, Armangue T, Planaguma J, Narsimhan R, Schob S, et al. Mouse model of anti-NMDA receptor post-herpes simplex encephalitis. *Neurol Neuroimmunol Neuroinflamm.* (2019) 6:e529. doi: 10.1212/NXI.0000000000000529
26. Baltagi SA, Shoykhet M, Felmet K, Kochanek PM, Bell MJ. Neurological sequelae of 2009 influenza A (H1N1) in children: a case series observed during a pandemic. *Pediatr Crit Care Med.* (2010) 11:179–84. doi: 10.1097/PCC.0b013e3181cf4652
27. Xu CL, Liu L, Zhao WQ, Li JM, Wang RJ, Wang SH, et al. Anti-N-methyl-D-aspartate receptor encephalitis with serum anti-thyroid antibodies and IgM antibodies against Epstein-Barr virus viral capsid antigen: a case report and one year follow-up. *BMC Neurol.* (2011) 11:149. doi: 10.1186/1471-2377-11-149
28. Solis N, Salazar L, Hasbun R. Anti-NMDA Receptor antibody encephalitis with concomitant detection of *Varicella zoster* virus. *J Clin Virol.* (2016) 83:26–8. doi: 10.1016/j.jcv.2016.08.292
29. Irani SR, Stagg CJ, Schott JM, Rosenthal CR, Schneider SA, Pettingill P, et al. Faciobrachial dystonic seizures: the influence of immunotherapy on seizure control and prevention of cognitive impairment in a broadening phenotype. *Brain.* (2013) 136(Pt. 10):3151–62. doi: 10.1093/brain/awt212
30. Li W, Wu S, Meng Q, Zhang X, Guo Y, Cong L, et al. Clinical characteristics and short-term prognosis of LGI1 antibody encephalitis: a retrospective case study. *BMC Neurol.* (2018) 18:96. doi: 10.1186/s12883-018-1099-z
31. Flanagan EP, Kotsenas AL, Britton JW, McKeon A, Watson RE, Klein CJ, et al. Basal ganglia T1 hyperintensity in LGI1-autoantibody faciobrachial dystonic seizures. *Neurol Neuroimmunol Neuroinflamm.* (2015) 2:e161. doi: 10.1212/NXI.0000000000000161
32. Heine J, Pruss H, Bartsch T, Ploner CJ, Paul F, Finke C. Imaging of autoimmune encephalitis–relevance for clinical practice and hippocampal function. *Neuroscience.* (2015) 309:68–83. doi: 10.1016/j.neuroscience.2015.05.037
33. Finke C, Pruss H, Heine J, Reuter S, Kopp UA, Wegner F, et al. Evaluation of cognitive deficits and structural hippocampal damage in encephalitis with leucine-rich, glioma-inactivated 1 antibodies. *JAMA Neurol.* (2017) 74:50–9. doi: 10.1001/jamaneurol.2016.4226
34. Gaspard N, Foreman BP, Alvarez V, Cabrera Kang C, Probasco JC, Jongeling AC, et al. New-onset refractory status epilepticus: etiology, clinical features, and outcome. *Neurology.* (2015) 85:1604–13. doi: 10.1212/WNL.0000000000001940

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REVIEW
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Maternal Antibody and ASD: Clinical Data and Animal Models

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Over the past several decades there has been an increasing interest in the role of environmental factors in the etiology of neuropsychiatric and neurodevelopmental disorders. Epidemiologic studies have shifted from an exclusive focus on the identification of genetic risk alleles for such disorders to recognizing and understanding the contribution of xenobiotic exposures, infections, and the maternal immune system during the prenatal and early post-natal periods. In this review we discuss the growing literature regarding the effects of maternal brain-reactive antibodies on fetal brain development and their contribution to the development of neuropsychiatric and neurodevelopmental disorders. Autoimmune diseases primarily affect women and are more prevalent in mothers of children with neurodevelopmental disorders. For example, mothers of children with Autism Spectrum Disorder (ASD) are significantly more likely to have an autoimmune disease than women of neurotypically developing children. Moreover, they are four to five times more likely to harbor brain-reactive antibodies than unselected women of childbearing age. Many of these women exhibit no apparent clinical consequence of harboring these antibodies, presumably because the antibodies never access brain tissue. Nevertheless, these maternal brain-reactive antibodies can access the fetal brain, and some may be capable of altering brain development when present during pregnancy. Several animal models have provided evidence that *in utero* exposure to maternal brain-reactive antibodies can permanently alter brain anatomy and cause persistent behavioral or cognitive phenotypes. Although this evidence supports a contribution of maternal brain-reactive antibodies to neurodevelopmental disorders, an interplay between antibodies, genetics, and other environmental factors is likely to determine the specific neurodevelopmental phenotypes and their severity. Additional modulating factors likely also include the microbiome, sex chromosomes, and gonadal hormones. These interactions may help to explain the sex-bias observed in neurodevelopmental disorders. Studies on this topic provide a unique opportunity to learn how to identify and protect at risk pregnancies while also deciphering critical pathways in neurodevelopment.

Keywords: brain-reactive antibodies, autism spectrum disorder, neurodevelopmental disorders, sex bias, gonadal hormones, sex chromosomes, microbiome

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INTRODUCTION

The increasing evidence of an immune mediated pathogenesis for neuropsychiatric and neurodevelopmental disorders has shifted the focus of epidemiologic studies to include the contribution of cytokines and brain-reactive antibodies. The brain was originally thought to be an immune privileged organ due to the presence of the blood brain barrier (BBB), a structure composed of endothelial cells knit together by tight junctions and supported by astrocytic endfeet (1). We now know that even though the BBB isolates the central nervous system (CNS) from factors in the blood, it is a dynamic semipermeable structure. Immune molecules including antibodies can access the CNS during both physiologic and pathologic states. Even though antibodies cannot cross the BBB and access brain tissue in healthy adults, these molecules may cross the BBB during *in utero* development when the BBB is immature and more permeable (2). Alternatively, antibodies can penetrate the adult brain when there is a BBB breach as occurs during inflammation (3–6) or at sites of limited BBB protection such as the choroid plexus. Factors affecting BBB integrity include: trauma, ischemia, stress, aging, antibodies, and specific agonists of endothelial cell receptors, such as cytokines, complement, and antibodies themselves (7–12). Once in the CNS, antibodies can lead to pathology if they recognize antigens expressed in the brain or spinal cord. In this review we discuss how maternal brain-reactive antibodies affect fetal brain development, contributing to the risk of neuropsychiatric and neurodevelopmental disorders. We focus on antibodies implicated in Autism Spectrum Disorder (ASD) and propose a role for the microbiome, sex chromosomes and gonadal hormones in determining the susceptibility to the effects of maternal antibody and the development of neurodevelopmental disorders.

Abbreviations: ASD, Autism Spectrum Disorder; BBB, Blood Brain Barrier; CNS, Central Nervous System; AD, Autoimmune disease; SLE, Systemic Lupus Erythematosus; NMO, Neuromyelitis Optica; LPS, lipopolysaccharide; NSPA, neuronal surface P antigen; NMDAR, N-methyl-D-aspartate receptors; DNRAb, SLE anti-DNA antibodies that cross-react with N-methyl-D-aspartate receptors; FcRn, neonatal Fc receptor; ADHD, Attention Deficit and Hyperactivity Disorders; DSM-V, Diagnostic and Statistical Manual of Mental Disorders Fifth Edition; ND, neurodevelopmental and neuropsychiatric disorders; NT, neurotypically developing; PPD, Pervasive Developmental Disorders; LD, Learning disabilities; TS, Tourette Syndrome; RA, rheumatoid arthritis; TPOAbs, Thyroid peroxidase antibodies; Abs, antibodies; P, post-natal day; IHC, Immunohistochemistry; NB, Nueroblastoma; WB, Western blot; LDH-A, lactate dehydrogenase A; LDH-B, lactate dehydrogenase B;YBX1, Y-box bonding protein 1; STIP1, stress-induced phosphoprotein 1; CRMP1, collapsing response mediator protein 1; CRMP2, collapsing response mediator protein 2; GDA, guanine deaminase; MSEL, Mullen Scales of Early Learning; VABS, Vineland Adaptive Behavioral Scales; ABC, Aberrant Behavioral Checklist; SVZ, subventricular zone; USVs, ultrasonic vocalizations; CNTNAP2, Contactin Associated Protein-Like 2; HEK, Human embryonic kidney cells; tGFP, turbo Green fluorescent protein; KO, Knockout; CDFE, cortical dysplasia-focal epilepsy; E, embryonic day; IV, intravenous; IP, intraperitoneal; SGZ, subgranular zone; VZ, ventricular zone; FCG, Four Core Genotype; Sry, testes determining gene; Y^-, Y chromosome with deleted Sry; TgSry, Sry transgene; GI, gastrointestinal; MIA, Maternal Immune Activation; SFB, Segmented filamentous bacteria.

BRAIN-REACTIVE ANTIBODIES

Antibodies that recognize CNS antigens are primarily detected in three settings: autoimmune disease (AD), paraneoplastic syndromes, and infectious diseases (13). Individuals with AD in which B cell tolerance is impaired can harbor brain-reactive antibodies with the development of neurological and neuropsychiatric disorders as seen in Systemic Lupus Erythematosus (SLE) (5, 14–20), celiac disease (21, 22), and Neuromyelitis Optica (NMO) (23–26). Due to the fact that the BBB sequesters brain antigen from the immune system, these brain-reactive antibodies may be produced against non-CNS antigens, but cross-react with structurally similar epitopes in the CNS. In paraneoplastic syndromes brain cross-reactive antibodies can result from an immune response to tumor antigens that are routinely expressed by brain cells but only by non-brain cells under pathologic states. These antibodies can trigger neurologic symptoms (27), a phenomenon that has been described in breast cancer (28, 29), testicular tumors (28), small-cell lung cancer (28, 30), ovarian teratoma (31, 32), and more (33–36). Finally, exposure to microbial antigens can stimulate the production of antibodies that cross-react with CNS antigens, a process known as molecular mimicry. Infection with HTLV-1 (37, 38), Trypanosoma brucei (39–41), and group A β-hemolytic streptococcus (42–47) has been shown to produce antibodies that cross-react with brain antigens and cause neurologic disorders.

The potential for pathology to arise from brain-reactive antibodies accessing the brain parenchyma depends on multiple factors. Vulnerability to the brain-reactive antibodies requires that the anti-brain antibody be present in the CNS at a time when the antigen is expressed. Furthermore, when a BBB breach is necessary for the antibody to penetrate the brain parenchyma, the nature of the BBB insult will restrict access to specific regions of the brain, determining whether the antibody will encounter its cognate antigen. For instance, lipopolysaccharide (LPS) causes a BBB breach in the hippocampus in mice (5) while epinephrine results in a BBB breach in the amygdala (4). Additionally, genetic variants and differences in protein expression between males and females (48–50) may also influence brain vulnerability to immune assault.

The symptoms and deficits observed in disorders caused by brain-reactive antibodies are dependent on the antigen recognized and its distribution in the brain. For example, antibodies to ribosomal P protein (anti-P antibodies) present in SLE patients cross-react with a brain antigen termed neuronal surface P antigen (NSPA) (19). While these antibodies bind to several regions of the mouse brain, when injected into the lateral cerebral ventricles they lead to smell alterations (51) and depression-like behavior (52), implicating the piriform cortex, the cingulate cortex, and the hippocampus. Furthermore, upon LPS-induced BBB breach, which enables access to the hippocampus, these antibodies cause memory impairment in mice (15). SLE anti-DNA antibodies that cross-react with N-methyl-D-aspartate receptors (NMDAR) termed DNRAb cause cognitive impairment (5) and an abnormal stress response (4) in mice, consistent with the function of NMDARs in the hippocampus and the amygdala, respectively. While NMDARs

are expressed in both the hippocampus and the amygdala, the regional selectivity of symptoms observed in the mouse models is determined by the agent used to compromise BBB integrity; cognitive impairment is seen only when LPS damages BBB integrity in the hippocampus while the abnormal stress response is seen with exposure to epinephrine and antibody penetration of the amygdala. This example highlights the importance of antigen distribution and the region of BBB breach.

MATERNAL BRAIN-REACTIVE ANTIBODIES AND FETAL BRAIN DEVELOPMENT

Brain-reactive antibodies have the potential to alter brain development *in utero* (53), resulting in damage that can be persistent (54) and lead to neurodevelopmental and neuropsychiatric disorders in the offspring. It has been proposed that AD-related impaired B cell tolerance in women can lead to the production of these antibodies. Indeed, ADs are more prevalent in mothers of children with neurodevelopmental and neuropsychiatric disorders including ASD (**Table 1**). A study by our group showed that mothers of children with ASD who had brain-reactive antibodies were significantly more likely to harbor anti-nuclear antibodies (53%) than those lacking brain-reactive antibodies (anti-nuclear antibody = 13.4%) and unselected women of child-bearing age (anti-nuclear antibody = 15%), suggesting a maternal predisposition to producing auto-antibodies. We also observed a significantly greater prevalence of AD in mothers of children with ASD who were also positive for brain-reactive antibodies [rheumatoid arthritis (RA) = 3.86%; SLE = 2.22%] compared to those lacking brain-reactive antibodies (RA = 1.45%; SLE = 0.16%) (67).

The BBB is immature during fetal development, presenting a uniquely vulnerable temporal window when antibodies can enter the CNS (2) while critical neurodevelopmental events are taking place. The antibodies that access the fetal brain are produced by the mother and are transported by the neonatal Fc receptor (FcRn) across the placenta into fetal circulation starting on week 13 of human gestation (68–70). Once maternal brain-reactive antibodies access the fetal circulation they may result in pathology if the timing of antigen expression also coincides with the period when the fetal BBB is permeable to IgG. Presence of maternal brain-reactive antibodies during pregnancy alone is, therefore, not enough to result in pathology, perhaps contributing to the fact that some mothers of neurotypically developing (NT) children also have anti-brain antibodies.

When assessing the potential for maternal brain-reactive antibodies to cause neurodevelopmental disorders we must also be mindful that the women harboring these antibodies will not necessarily present with neurological deficits resulting from antibody exposure as their BBB is likely to be intact or the impact of the antibody may be developmentally determined. Moreover, the deficits caused by *in utero* antibody exposure may be transient or long-lasting, and consequently, not all children born to mothers with brain-reactive antibodies will present post-natally with detectable symptoms. Transient insults may be compensated for by plasticity mechanisms in the brain during gestation or post-natally. Furthermore, it is possible for deficits to be present only while the pathogenic antibody has access to the brain parenchyma, which is limited after birth by the maturation of the BBB (2) and the disappearance of maternal antibodies in the circulation of newborn infants (71). Lastly, symptomatology from the neurodevelopmental effects of maternal brain-reactive antibodies may appear later in life or may become apparent only if other stressors are present (72).

MATERNAL BRAIN-REACTIVE ANTIBODIES AND ASD

ASD are a group of neurodevelopmental conditions characterized by impaired communication and social interactions, repetitive behaviors, and restricted interests or activities (DSM-V) (73). They are four times more likely to be diagnosed in males compared to females. Both prevalence and incidence of ASD are increasing, with a current estimate of 1 in 59 children being affected (74). The etiology of ASD is not completely understood; hundreds of genes have been associated with ASD (75, 76) but these account for just 10–20% of the diagnosed cases (77). Furthermore, twin studies indicate that only 37% of the susceptibility to ASD is due to genetic heritability (78). These data suggest that environmental factors also play an important role in determining the susceptibility to ASD. Maternal brain-reactive antibodies present *in utero* represent a potential environmental risk factor for ASD. Several groups have identified brain-reactive antibodies in mothers of children with ASD which are either absent or found at lower frequency in mothers of unaffected children (**Table 2**). Dalton et al. (79) showed that, when injected into pregnant mice, brain-reactive serum from a mother of a child with autism and a child with severe specific language disorder led to decreased exploration, deficient motor coordination, and altered cerebellar metabolites in the offspring compared to the offspring of mice given sera from mothers of NT children. In a study in which blood samples were collected mid-pregnancy, Croen et al. (80) suggested a direct pathogenic role for the antibodies. A significantly higher prevalence of reactivity to proteins from brain lysates of 39 and 73 kDa was detected in mothers of children with ASD compared to the general population control group. Moreover, this pattern of reactivity was seen in mothers of children with early onset ASD.

Some of these maternal brain-reactive antibodies have been found to have antigenic specificity for proteins with potential neurodevelopmental roles including: lactate dehydrogenase A and B (LDH-A, LDH-B) (37 kDa band), Y-box bonding protein 1 (YBX1) (39 kDa band), stress-induced phosphoprotein 1 (STIP1) (upper 73 kDa band), collapsing response mediator protein 1 and 2 (CRMP1, CRMP2) (lower 70 kDa band), and guanine deaminase (GDA) (44 kDa band) (83, 84). Maternal reactivity to LDH alone or in combination with reactivity to CRMP1/CRMP2/STIP1 has been associated with a greater risk of ASD. Reactivity to LDH, STIP1, and CRMP1 together (the 37 and 73 kDa combined bands) was the most specific pattern for ASD

TABLE 1 | Family history of autoimmune diseases and increased risk of neurodevelopmental/neuropsychiatric disorders.

	Associations with autoimmune disease (AD)	Prevalence/incidence of Neurodevelopmental/neuropsychiatric disorders (ND)		Family history of AD		ND sex bias linked to AD	References
		AD offspring	Control offspring	ND	NT		
Developmental problems	Maternal SLE	Hyperactivity = 13.1% Attention problems = 15.7% Reading difficulties = 21.6%	Hyperactivity = 1.3% Attention problems = 6% Reading difficulties = 9.3%			Male bias	(55)
Learning Disabilities (LD)	Increased risk of LD and maternal anti-Ro/La Abs [OR = 5.74 (95% CI, 1.39–23.74)] and SLE disease flares [OR = 9.43 (95% CI, 1.32–67.24)] during pregnancy	26%	7%			Male bias	(56)
	Maternal SLE and increased risk of impairments in learning and memory [OR = 3.45, 95% CI of OR (1.25, 9.09), $P = 0.02$]	54.9%	30.4%				(57)
Tourette Syndrome (TS)	Maternal AD and increased incidence of TS [IRR = 1.22 (95% CI, 1.01–1.48)]	2.25 per 10,000 person years	1.86 per 10,000 person years			Male bias	(58)
PDD	1st degree relative with a history of AD Family history of Hashimoto's thyroiditis and rheumatic fever			30.7%	11.9%		(59)
ADHD	Elevated maternal TPOAbs during pregnancy and increased risk of ADHD [OR = 1.77 (95% CI, 1.15–2.72)						(60)
ASD	Maternal RA and increased incidence of ASD [IRR = 1.70 (95% CI, 1.07–2.54)] Maternal celiac disease and increased incidence of ASD [IRR = 2.97 (95% CI, 1.27–5.75] Family history of type 1 diabetes and increased incidence of ASD [IRR = 1.78 (95% CI, 1.16–2.61)]						(61)
	AD in 1st degree relative and increased risk of ASD [OR 6.0] Maternal AD and increased risk of ASD [OR = 8.8]			1st degree relative AD = 21% Maternal AD = 16% RA = 46%	1st degree relative AD = 4% Maternal AD = 2% RA = 26%		(62)
	Maternal psoriasis and increased risk of ASD [OR = 2.7 (95% confidence interval, 1.3–5.8)]			Psoriasis = 2.7%	Psoriasis = 1.0%		(63)
	Family history of AD and increased risk of ASD [OR = 6, 95% CI, 2.5–14.1)]			AD = 40%	AD = 10%		(64)
				Family history AD = 45%	Family history AD = 10%		(65)
	Maternal SLE and increased risk of ASD [OR = 2.19 (95% CI 1.09–4.39)]	1.4%	0.6%				(66)

AD, autoimmune disease; ND, Neurodevelopmental/neuropsychiatric disorders; NT, Neurotypical development; SLE, Systemic lupus erythematosus; LD, learning disabilities; Abs, antibodies; TS, Tourette Syndrome; PDD, Pervasive Developmental Disorders; ADHD, Attention deficit/hyperactivity disorder; TPOAbs, thyroid peroxidase antibodies; ASD, Autism Spectrum Disorder; RA, Rheumatoid arthritis.

TABLE 2 | Maternal brain-reactive antibodies linked to ASD.

Target	Reactivity	Phenotypic associations in humans	References
Cerebellar Purkinje cells and brainstem neurons	Adult rat and P1 mouse brains by IHC Binding to NB-1 cells (cells derived from a human neuroblastoma)	Serum obtained from a mother of a child with ASD and a child with a language disorder	(79)
LDH-A, LDH-B	37 kDa band on WB using human and rhesus macaque fetal brain proteins	ASD with behavioral regression Within individuals with ASD, 1. Abnormal sleep/wake cycle 2. Deficits in verbal and non-verbal language acquisition 3. Increased stereotypical behaviors on the ABC	(81–86)
STIP1 (target for upper band CRMP1, CRMP2 (target for lower band)	73 kDa band on WB using human and rhesus macaque fetal brain proteins	Within individuals with ASD, 1. Verbal language deficits 2. Delayed onset of social smile	(82–86)
LDH reactivity in combination with STIP1 or STIP1/CRMP1	37/73 kDa band combination on WB using human and rhesus macaque fetal brain proteins	1. Pattern only observed in mothers of children with ASD 2. ASD with behavioral regression 3. Greater total cerebral volume Within individuals with ASD, 4. Lower score for expressive language measured by MSEL 5. Increased stereotypical behaviors on the ABC	(81–83, 85–87)
YBX1	39 kDa band on WB using human and rhesus macaque fetal brain proteins	Early onset ASD	(80, 82–86)
YBX1 reactivity in combination with STIP1 or STIP1/CRMP1	39 kDa/73 kDa band combination on WB using human and rhesus macaque fetal brain proteins	1. Early onset ASD 2. Decreased motor skills scores measured with VABS 3. Increased irritability on the ABC compared to children with ASD born to mothers without these reactivities	(80, 82, 83, 85, 86)
Cypin or GDA	44 kDa band on WB using rhesus macaque fetal brain proteins		(83, 84)
Not identified	36 kDa/39 kDa band combination on WB with human fetal and rat embryonic brain proteins	ASD with developmental regression.	(88)
Not identified	Low molecular weight bands (∼20–25 kDa) High molecular weight band (larger than 250 kDa) on WB using fetal rat brain proteins		(89)
Yo	Immunoblot with recombinant protein		(90)
Amphiphysin	Immunoblot with recombinant protein		(90)
Caspr2	Isolation and cloning of single human memory antigen-specific B cells Live cell-based assay using HEK-293 cells expressing tGFP-Caspr2 Binding to adult wild type but not to adult CNTNAP2 KO mouse brain by IHC		(91)

P, ponstnatal day; IHC, Immunohistochemistry; NB, neuroblastoma; ASD, Autism Spectrum Disorder; WB, Western blot; LDH-A, lactate dehydrogenase A; LDH-B, lactate dehydrogenase B; STIP1, stress-induced phosphoprotein 1; CRMP1, CRMP2, collapsing response mediator protein 1 and 2; MSEL, Mullen Scales of Early Learning; NT, Neurotypical development; YBX1, Y-box bonding protein 1; VABS, Vineland Adaptive Behavioral Scales; ABC, Aberrant Behavioral Checklist; GDA, Guanine deaminase; HEK, Human Embryonic Kidney cells; tGFP, Turbo green fluorescent protein; Caspr2, Contactin-associated protein-like 2; CNTNAP2, Contactin-associated protein-like 2 gene; KO, Knockout.

as it was detected in mothers of children with ASD but not in mothers of NT children (81, 83). Furthermore, presence of this antibody combination was associated with an increased risk of behavioral regression in ASD (81) and impairments in expressive language (82).

Animal studies have shown that exposure to maternal brain-reactive antibodies *in utero* can permanently alter the brain during development and cause sustained behavioral and cognitive deficits akin to those observed in ASD (**Table 3**). Martínez-Cerdeño et al. (96) and Ariza et al. (97) used a single

TABLE 3 | Animal studies demonstrating that *in utero* exposure to maternal brain-reactive antibodies can permanently alter the brain, leading to behavioral and cognitive deficits.

Target	Animal model	Characteristics	References
Cerebellar Purkinje cells and brainstem neurons	Passive transfer: daily maternal serum injections into pregnant mice from E10 to E17	Decreased exploration Altered motor coordination Cerebellar metabolite abnormalities.	(79)
Unknown	Passive transfer: IV injections of pooled maternal IgG to pregnant rhesus macaque on gestation days 27, 41 and 55	Hyperactivity Increased stereotypies	(92)
LDH/STIP1/CRMP1 (antigens of molecular weights 37 and 73 kDa)	Passive transfer: single IV injection of purified maternal IgG into pregnant mice on E12	Delayed pre-weaning motor and sensory development. Increased number of USVs on P8 Males had a longer total USV duration on P8 Increased anxiety-like behaviors in males Slightly shorter social interaction in males	(93)
	Passive transfer: IV maternal IgG injection into rhesus macaque throughout pregnancy	Aberrant social behaviors Enlarged brain volume due to increases in white matter in male offspring.	(94)
	Single intraventricular maternal IgG injection into E14 mouse embryos	Increased repetitive behaviors measured as digging in the marble test and grooming Impaired social interactions	(95)
	Single intraventricular maternal IgG injection into E14 or E16 mouse embryos	Greater number of proliferating stem cells in the SVZ of the neocortex and ganglionic eminence Increased adult brain size and weight Increased adult cortical neuron some volume	(96)
	Single intraventricular maternal IgG injection into E14 mouse embryos	Decreased basal dendritic arborization in layer V pyramidal neurons of the frontal cortex Reduced the dendritic spine number and density in several brain regions	(97)
	Endogenous production: female mice were immunized prior to pregnancy with antigenic peptides recognized by anti- LDH/STIP1/CRMP1 antibodies.	Impaired social interactions Impaired social communication measured by USVs neonatally and as adults. Increased repetitive behaviors measured as grooming	(98)
Unknown	Passive transfer: daily IP injections of pooled maternal IgG to pregnant mice from E13 to E18.	Hyperactivity Increased anxiety Impaired social interactions Increased IL-12 levels on E16 and microglia activation on E18 fetal brains.	(99)
	Passive transfer: daily IP injections of pooled maternal IgG to pregnant mice from E13 to E18.	Greater cell proliferation in the SVZ and SGZ post-natally. Decreased cortical cell survival post-natally.	(100)
Caspr2	Passive transfer: single IV injection of anti-Caspr2 IgG to pregnant mice on E13.5	Male fetuses: 1. Thinner cortical plate 2. Fewer proliferating cells in the VZ 3. Reduced number of neurons in the entorhinal cortex Adult males: 1. Decreased number of GABAergic neurons in the hippocampus 2. Decreased dendritic arborization and spine density in CA1 pyramidal neurons 3. Increased stereotypic behaviors: increased digging measured as digging in the marble test 4. Impaired flexible learning 5. Impaired social interactions	(91)
DNA and NMDAR	Endogenous production: female mice were immunized prior to pregnancy with a peptide mimetope of DNA. Passive transfer: single IV injection of NMDAR reactive IgG on E14 to pregnant mice	Fetuses: 1. Increased cortical cell death and proliferation 2. Thinner cortical plate Adults: 1. Decreased cortical neuron size 2. Decreased cortical volume 3. Cognitive impairments in males	(101)
	Endogenous production: female mice were immunized prior to pregnancy with a peptide mimetope of DNA.	Increased female fetal death rate	(102)

E, Embryonic day; IV, intravenous; LDH, lactate dehydrogenase; STIP1, stress-induced phosphoprotein 1; CRMP, collapsing response mediator protein 1; SVZ, subventricular zone; USVs, ultrasonic vocalizations; P, ponstnatal day; IP, intraperitoneal; SGZ, subgranular zone; Caspr2, Contactin-associated protein-like 2; VZ, ventricular zone; NMDAR, N-Methyl-D-aspartate receptor.

intraventricular embryonic injection model to assess the effects of brain-reactive antibodies recognizing LDH/STIP1/CRMP1 (antigens of molecular weights 37 and 73 kDa) on fetal brain development. These antibodies stimulated the proliferation of stem cells in the subventricular zone (SVZ) of the neocortex and ganglionic eminence, increased adult brain size and weight, and enlarged adult cortical neuron volume (96). Additionally, anti-LDH/STIP1/CRMP1 antibodies decreased basal dendritic arborization in layer V neurons of the frontal cortex and reduced the dendritic spine number and density in several brain regions (97). Complementing these studies, mice exposed *in utero* to maternal brain-reactive antibodies recognizing LDH/STIP1/CRMP1 showed ASD-like characteristics including increased anxiety-like behaviors (93), impaired social interactions (95), longer bouts of spontaneous grooming (95), increased digging (95), and delayed motor and sensory development (93).

A limitation of these animal studies is the timing of exposure to the maternal brain-reactive antibodies. Maternal brain-reactive antibodies associated with ASD in humans are likely to be present throughout the pregnancy. These studies did not simulate these conditions; they used a single injection into the pregnant mice at mid-gestation or a single intraventricular injection into the embryos. To address this, Jones et al. (98) completed a study in which female mice were immunized prior to pregnancy with antigenic peptides recognized by anti-LDH/STIP1/CRMP1 antibodies. The offspring mice exposed to endogenous maternal anti-LDH/STIP1/CRMP1 antibodies displayed fewer social interactions as juveniles and adults, increased repetitive behaviors/stereotypies assessed through the number and length of grooming bouts, and impaired social communication measured by ultrasonic vocalizations (USVs) neonatally and as adults.

In other studies identifying pathologic maternal brain-reactive antibodies, Singer et al. (88) found that, compared to mothers of NT children, mothers of children with ASD have a significantly higher prevalence of antibodies reactive to a 36 kDa protein present in rat embryonic and human fetal brain. They also noted a trend for a higher prevalence of antibodies recognizing human fetal proteins at 39 kDa in mothers of children with ASD compared to mothers of NT children. Presence of either of these reactivities was significantly associated with behavioral regression in children with ASD. Intraperitoneal administration of the purified maternal ASD-IgG to pregnant mice led to hyperactivity, increased anxiety, and shorter social interactions in the adult offspring relative to the offspring of pregnant mice given IgG from mothers of NT children or saline (99). Preliminary fetal brain studies suggested a role for microglia and IL-12 in the pathological mechanism of the ASD-IgG induced behavioral irregularities (99). Further assessment of the pathological mechanism of these antibodies showed greater cell proliferation in the subventricular and subgranular zones and decreased post-natal day (P)1-born cell density, suggesting reduced survival, in layers 2–4 of the frontal and parietal cortex (100). Due to the similarity in antigen size and the association with behavioral regression, it is possible that the samples from the Braunschweig and Singer studies contain antibodies that recognize the same proteins at 36–39 and 73 kDa, and that these antibodies represent contributors to ASD risk in the general population. Determining the antigenicity of the antibodies identified by Singer et al. (88) will be necessary to resolve this question.

Animal studies of the pathogenic role of ASD-IgG have generally used IgG that was pooled from several mothers of children with ASD or endogenous polyclonal antibody following an immunization protocol. Thus, it has not been possible to identify the antibodies that are pathogenic from those that are not. Furthermore, specific ASD-like characteristics in the animal studies may result from exposure to distinct monoclonal antibodies. There is also likely to be a different proportion of potentially pathogenic antibodies in the pooled sample compared to the composition present in each of the mothers. As a result, the effects of antibodies at low concentration might be obscured by those at high concentration or they may not be detectable because the threshold concentration of antibody necessary to produce pathology may not have been reached. Moreover, the studies discussed above do not definitively identify the targeted antigen as it remains possible that the critical antibodies bind not only to the identified antigens (most of which are intracellular) but also cross-react with a neuronal membrane antigen. We addressed this concern by developing a protocol to generate monoclonal brain-reactive antibodies from mothers of children with ASD and a brain-reactive serology.

One of the monoclonal antibodies that we generated recognizes the extracellular domain of Caspr2, a protein encoded by the gene Contactin Associated Protein-Like 2 (CNTNAP2). Caspr2 is a cell-adhesion molecule expressed in the spines, dendrites, axons, and soma of neurons (103, 104). Both rare and common variants of CNTNAP2 have been linked to an increased risk of ASD or ASD-related endophenotypes including language delay and developmental language disorders (105–115). Furthermore, CNTNAP2 deficient mice exhibit ASD-like phenotypes including increased repetitive behaviors, and impaired communication and social interactions (116). The CNTNAP2 deficient mice also suffer from seizures, show neuronal migration abnormalities and have ectopic neurons in the corpus callosum (116), similar to cortical dysplasia-focal epilepsy (CDFE) syndrome, a syndromic form of ASD associated with mutant CNTNAP2 (114).

Given the link between mutations in CNTNAP2 and ASD in human pedigrees and the presence of ASD-like phenotypes in CNTNAP2 deficient mice, we asked whether exposure to monoclonal anti-Caspr2 antibody (C6) *in utero* leads to ASD-like characteristics in mice (91). Indeed, *in utero* C6 exposure led to a thinner cortical plate and fewer proliferating cells in the ventricular zone, and to a reduction in the number of neurons in the entorhinal cortex and in the number of GABAergic neurons in the hippocampus of adults. We also observed decreased dendritic arborization and a reduced spine density in CA1 pyramidal neurons in adult mice exposed to C6 *in utero* when compared to the controls. Finally, these mice showed ASD-like behavioral abnormalities such as stereotypic behaviors, impaired flexible learning, and impaired social interactions. Interestingly, effects of C6 were only detected in male mice.

MATERNAL BRAIN-REACTIVE ANTIBODIES AND SEX-BIAS

Neuropsychiatric conditions often display a bias for one sex over the other. Neuropsychiatric conditions diagnosed earlier in life are more frequently diagnosed in males while those manifesting during puberty or later in life show a female preponderance. For example, there is a male bias in ASD, ADHD, dyslexia, Tourette Syndrome, and learning disabilities [reviewed by (117)]. Conversely, anorexia nervosa and internalizing disorders such as depression (118) and anxiety are more prevalent in females (119). Neuropsychiatric conditions that are diagnosed earlier in life have been proposed to have a neurodevelopmental origin (119). Given the unique conditions during pregnancy that allow for the *in utero* environment including maternal antibodies and cytokine levels to influence development, it is reasonable that early-onset neuropsychiatric disorders are more frequent in children of mothers with AD (**Table 1**).

A sex-bias has indeed been described in multiple studies of the effects of maternal brain-reactive antibodies on development and behavior. For example, we observed a significant male bias for all the fetal brain developmental and adult behavioral effects of the C6 anti-Caspr2 antibody (91). Females exposed to C6 *in utero* were not affected while males developed disrupted brain anatomy and ASD-relevant behaviors. The animal studies of maternal anti-LDH/STIP1/CRMP antibodies have also noted a male-bias in ASD-like traits including impaired communication and social interactions (93, 98). Lastly, Wang et al. (102) observed a significantly higher rate of fetal death after embryonic day (E) 15 in female offspring exposed to SLE DNRAb compared to males. While females had a greater fetal death risk, males in this model were born with cognitive impairment (101). This difference between the sexes suggests that sex-dependent factors play a role in determining not only the impairments resulting from maternal brain-reactive antibody exposure but also the severity of the outcomes.

Sex chromosomes and gonadal hormones may influence the susceptibility to maternal brain-reactive antibodies. Sex chromosome genes contribute to sexual dimorphisms, including sex-specific patterns of brain development and function, independently from gonadal hormone influences (50, 120). For example, sex chromosomes have been implicated in the density of vasopressin fibers (121), the number of tyrosine hydroxylase expressing neurons (120, 122), social interactions (123, 124), aggression (125–127), and anxiety (128). Genes found in "sex specific" regions in the X and the Y chromosomes may account for some of these sexual dimorphisms. These genes are highly expressed in the brain (48, 50) and show expression, spatial, and temporal differences between sexes (50). Furthermore, sex differences may be in part explained by gene dosage as some genes on the X chromosome escape inactivation (129–131), and X chromosome imprinting can affect gene expression in the brain (132, 133). Indeed, X chromosome imprinting has been associated with social impairment in Turner syndrome (134). Higher expression of these sex chromosome genes may be protective if they encode the antigens recognized by the maternal brain-reactive antibodies when binding of the antibody leads to protein internalization and partial loss of function but is only pathogenic if protein expression falls below a certain threshold. Conversely, if antibody interaction with its cognate antigen induces cell signaling cascade activation or apoptosis, then the sex with higher expression would be at an increased risk for developing the antibody-induced phenotypes. Furthermore, temporal differences in gene expression between sexes may be important as antibody exposure must coincide with this period in order to cause pathology. Moreover, the effects of sex chromosome genes on the susceptibility to maternal brain-reactive antibodies may be indirect if the proteins encoded modulate the expression or activity of the proteins recognized by the antibodies. For instance, the DNRAb mediated female fetal loss may be in part due to sex chromosome genes acting as regulating factors and contributing to the higher expression of the NMDAR subunit NR2A in the female brainstem by E17 (102).

Sex hormones also influence brain development, aiding normal maturation of the fetal brain or altering normal development. Estrogen has neurotrophic and neuroprotective functions including modulation of neuronal apoptosis, migration, and spinogenesis and neurite growth (135–140). Estrogen administration during fetal development leads to masculinization of mouse neural pathways and behaviors (141, 142). Human fetal testosterone has been linked to narrow interests (143) and greater impairments in social skills and empathy in offspring (143–145). Additionally, treatment with estrogen reversed or mitigated some of the ASD-relevant behavioral phenotypes in animal models of ASD, importantly, CNTNAP2 mutant zebrafish (146) and Reeler heterozygous mice (147). As estrogen treatment showed a decrease in phenotypic behavior, these data suggest that estrogen may have a protective role in ASD and could therefore account for the lower prevalence in females. Overall, gonadal hormones modulate processes in brain development and maturation that could lead to compensation for or exacerbation of the pathologic effects of maternal brain-reactive antibodies. The specific effects of individual gonadal hormones are likely to depend on the neural pathways affected by the antibodies.

Given the sex-bias of neurodevelopmental and neuropsychiatric disorders in humans, understanding what causes maternal brain-reactive antibodies to affect preferentially one sex over the other will aid in our understanding of the pathological mechanisms of these conditions while also providing information that could lead to the discovery of new treatments. Of note, exposure to gonadal hormones *in utero* in litter-bearing animals differs from that in humans due to the "intrauterine position phenomenon." Fetuses between two males will be exposed to higher levels of testosterone while fetuses between two females will be exposed to higher levels of estrogen [reviewed by (148)]. Consequently, post-natal sexually dimorphic characteristics including brain anatomy and behavior are influenced by fetal position [reviewed by (148)]. Nonetheless, the importance of gonadal hormones can be addressed in studies in which these are administered to neonates. Alternatively, treatment with gonadal hormone receptor agonists or antagonists, and the use of gonadal hormone receptor knockout mice can not only help us to identify which hormones play a role in the sex-bias that is observed but can also lead to the identification of the specific

molecular mechanisms involved. The "Four Core Genotypes" (FCG) mouse model offers the unique opportunity to isolate sex chromosome from gonadal hormone contributions to sex-bias. This mouse strain combines two mutations that allow for gonadal determination to be independent from sex chromosome complements. Specifically, the testes determining gene (Sry) was deleted from the Y chromosome (Y^-) and a Sry transgene (TgSry) was inserted into autosomal chromosome 3, resulting in four genotypes: gonadal females with XX or XY^-, and gonadal males with XY^- TgSry or with XX TgSry (121, 149). This model has been informative in understanding the sex bias in autoimmune diseases such as SLE and multiple sclerosis (150).

ASD AND THE MICROBIOME

The gut microbiome is another environmental factor proposed to exert significant modulating effects on ASD susceptibility. Gastrointestinal (GI) disturbances, including increased intestinal permeability (151, 152) and inflammatory bowel disease (153), represent a common comorbidity in individuals with ASD (154, 155). Furthermore, there is a strong correlation between GI complaints and ASD symptom severity (156, 157). Given the effect of the gut microbiota on behavior [(158–164); reviewed by (165)], brain development [(3); reviewed by (165)] and brain gene expression in mice (158, 162–164, 166, 167), alterations of the gut microbiota may not only lead to an increase in the prevalence of GI disturbances in ASD but also promote ASD susceptibility. Indeed, individuals with ASD can have an altered gut microbiota composition (156, 168–173).

The gut microbiota may act in concert with maternal brain-reactive antibodies, genetic variants and sex-specific factors to modulate ASD susceptibility pre- and post-natally. In the model of maternal immune activation (MIA), specific maternal gut microbiota are necessary for the development of MIA-associated behavioral phenotypes and neurodevelopmental abnormalities. Segmented filamentous bacteria (SFB) -specific T_H17 cells are stimulated by dendritic cells primed by poly (I:C) to produce high levels of IL-17a (174), a key cytokine for ASD-like phenotype induction in the MIA model (175, 176). Furthermore, during fetal development, metabolites produced by the maternal gut microbiota can alter BBB tight junction protein expression thus modifying BBB permeability. Offspring of germ-free mice have a decreased expression of occludin and claudin-5 which contributes to the increased BBB permeability observed from the fetal stage until adulthood (3). This increased BBB permeability makes the offspring more susceptible to the neurological effects of immune molecules including antibodies as these are then more likely to access the brain parenchyma (3). Finally, shifts in offspring microbiota composition can be induced by factors in the *in utero* environment and contribute to the development of ASD-like behavioral deficits. For example, the offspring of poly (I:C) treated mice have an altered gut microbiota diversity which leads to altered serum metabolites and increased IL-6 and gut permeability (177). Hsiao et al. (177) propose that the increased IL-6 expression alters tight junction protein expression, leading to increased gut permeability, and leaking of harmful metabolites into systemic circulation. Post-natal treatment of the offspring with *Bacteroides fragilis* reversed some of the ASD-like behavioral phenotypes and improved gut barrier permeability, possibly by restoring IL-6 expression, which in turn leads to a partial correction of tight junction protein expression (177).

CONCLUSIONS

The association between maternal brain-reactive antibodies and the pathogenesis of neurodevelopmental disorders has been well-established by both epidemiologic and animal studies. Maternal autoimmune disease and brain-reactive antibodies have been shown to increase the risk of neurodevelopmental

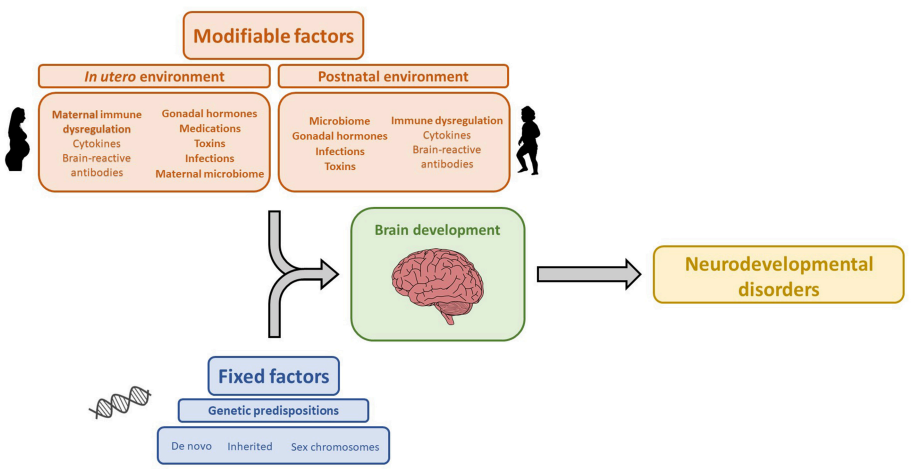

FIGURE 1 | Genetic and environmental factors that influence brain development and contribute to the etiology of neurodevelopmental disorders. The icon images included in this Figure were obtained from openclipart.org, and are images in the Public Domain.

disorders. Moreover, animal studies have shown that *in utero* exposure to maternal brain-reactive antibodies is sufficient to permanently alter brain anatomy and cause aberrant cognition or behavior mimicking certain neurodevelopmental syndromes. Specific neurodevelopmental disorders and the severity of symptomatology are likely determined by an interplay between genetics and environmental risk factors including maternal brain-reactive antibody, maternal cytokines, gonadal hormones, and the microbiome (**Figure 1**). As the prevalence of neurodevelopmental disorders has been significantly increasing (178), research on the *in utero* environment, including maternal brain-reactive antibodies, is of great biomedical importance. Identifying potentially pathogenic antibodies and understanding their mechanisms of fetal brain injury provide an opportunity to detect and protect fetuses at risk.

AUTHOR CONTRIBUTIONS

AG-G contributed to the concept and writing of the manuscript. BD contributed to the concept, writing, and reviewing of the manuscript.

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REFERENCES

1. Goldstein GW. Endothelial cell-astrocyte interactions. A cellular model of the blood-brain barrier. *Ann N Y Acad Sci.* (1988) 529:31–9. doi: 10.1111/j.1749-6632.1988.tb51417.x
2. Kowal C, Athanassiou A, Chen H, Diamond B. Maternal antibodies and developing blood-brain barrier. *Immunol Res.* (2015) 63:18–25. doi: 10.1007/s12026-015-8714-5
3. Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Tóth M, et al. The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med.* (2014) 6:263ra158. doi: 10.1126/scitranslmed.3009759
4. Huerta PT, Kowal C, DeGiorgio LA, Volpe BT, Diamond B. Immunity and behavior: antibodies alter emotion. *Proc Natl Acad Sci USA.* (2006) 103:678–83. doi: 10.1073/pnas.0510055103
5. Kowal C, DeGiorgio LA, Nakaoka T, Hetherington H, Huerta PT, Diamond B, et al. Cognition and immunity; antibody impairs memory. *Immunity.* (2004) 21:179–88. doi: 10.1016/j.immuni.2004.07.011
6. Tsao N, Hsu HP, Wu CM, Liu CC, Lei HY. Tumour necrosis factor-alpha causes an increase in blood-brain barrier permeability during sepsis. *J Med Microbiol.* (2001) 50:812–21. doi: 10.1099/0022-1317-50-9-812
7. Ballabh P, Braun A, Nedergaard M. The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol Dis.* (2004) 16:1–13. doi: 10.1016/j.nbd.2003.12.016
8. Matsueda Y, Arinuma Y, Nagai T, Hirohata S. Elevation of serum anti-glucose-regulated protein 78 antibodies in neuropsychiatric systemic lupus erythematosus. *Lupus Sci Med.* (2018) 5:e000281. doi: 10.1136/lupus-2018-000281
9. Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, et al. Blood-brain barrier breakdown in the aging human hippocampus. *Neuron.* (2015) 85:296–302. doi: 10.1016/j.neuron.2014.12.032
10. Thom G, Hatcher J, Hearn A, Paterson J, Rodrigo N, Beljean A, et al. Isolation of blood-brain barrier-crossing antibodies from a phage display library by competitive elution and their ability to penetrate the central nervous system. *MAbs.* (2018) 10:304–14. doi: 10.1080/19420862.2017.1409320
11. Wylezinski LS, Hawiger J. Interleukin 2 activates brain microvascular endothelial cells resulting in destabilization of adherens junctions. *J Biol Chem.* (2016) 291:22913–23. doi: 10.1074/jbc.M116.729038
12. Yoshio T, Okamoto H, Hirohata S, Minota S. IgG anti-NR2 glutamate receptor autoantibodies from patients with systemic lupus erythematosus activate endothelial cells. *Arthritis Rheum.* (2013) 65:457–63. doi: 10.1002/art.37745
13. Diamond B, Honig G, Mader S, Brimberg L, Volpe BT. Brain-reactive antibodies and disease. *Annu Rev Immunol.* (2013) 31:345–85. doi: 10.1146/annurev-immunol-020711-075041
14. Arinuma Y, Yanagida T, Hirohata S. Association of cerebrospinal fluid anti-NR2 glutamate receptor antibodies with diffuse neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum.* (2008) 58:1130–5. doi: 10.1002/art.23399
15. Bravo-Zehnder M, Toledo EM, Segovia-Miranda F, Serrano FG, Benito MJ, Metz C, et al. Anti-ribosomal P protein autoantibodies from patients with neuropsychiatric lupus impair memory in mice. *Arthritis Rheumatol.* (2015) 67:204–14. doi: 10.1002/art.38900
16. DeGiorgio LA, Konstantinov KN, Lee SC, Hardin JA, Volpe BT, Diamond B. A subset of lupus anti-DNA antibodies cross-reacts with the NR2 glutamate receptor in systemic lupus erythematosus. *Nat Med.* (2001) 7:1189–93. doi: 10.1038/nm1101-1189
17. Faust TW, Chang EH, Kowal C, Berlin R, Gazaryan IG, Bertini E, et al. Neurotoxic lupus autoantibodies alter brain function through two distinct mechanisms. *Proc Natl Acad Sci USA.* (2010) 107:18569–74. doi: 10.1073/pnas.1006980107
18. Harrison MJ, Ravdin LD, Lockshin MD. Relationship between serum NR2a antibodies and cognitive dysfunction in systemic lupus erythematosus. *Arthritis Rheum.* (2006) 54:2515–22. doi: 10.1002/art.22030
19. Matus S, Burgos PV, Bravo-Zehnder M, Kraft R, Porras OH, Farías P, et al. Antiribosomal-P autoantibodies from psychiatric lupus target a novel neuronal surface protein causing calcium influx and apoptosis. *J Exp Med.* (2007) 204:3221–34. doi: 10.1084/jem.20071285
20. Omdal R, Brokstad K, Waterloo K, Koldingsnes W, Jonsson R, Mellgren SI. Neuropsychiatric disturbances in SLE are associated with antibodies against NMDA receptors. *Eur J Neurol.* (2005) 12:392–8. doi: 10.1111/j.1468-1331.2004.00976.x
21. Alaedini A, Okamoto H, Briani C, Wollenberg K, Shill HA, Bushara KO, et al. Immune cross-reactivity in celiac disease: anti-gliadin antibodies bind to neuronal synapsin I. *J Immunol.* (2007) 178:6590–5. doi: 10.4049/jimmunol.178.10.6590
22. Hadjivassiliou M, Aeschlimann P, Strigun A, Sanders DS, Woodroofe N, Aeschlimann D. Autoantibodies in gluten ataxia recognize a novel neuronal transglutaminase. *Ann Neurol.* (2008) 64:332–43. doi: 10.1002/ana.21450
23. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med.* (2005) 202:473–7. doi: 10.1084/jem.20050304
24. Mader S, Lutterotti A, Di Pauli F, Kuenz B, Schanda K, Aboul-Enein F, et al. Patterns of antibody binding to aquaporin-4 isoforms in neuromyelitis optica. *PLoS ONE.* (2010) 5:e10455. doi: 10.1371/journal.pone.0010455
25. Hinson SR, McKeon A, Fryer JP, Apiwattanakul M, Lennon VA, Pittock SJ. Prediction of neuromyelitis optica attack severity by quantitation of complement-mediated injury to aquaporin-4-expressing cells. *Arch Neurol.* (2009) 66:1164–7. doi: 10.1001/archneurol.2009.188
26. Zhang H, Bennett JL, Verkman AS. *Ex vivo* spinal cord slice model of neuromyelitis optica reveals novel immunopathogenic mechanisms. *Ann Neurol.* (2011) 70:943–54. doi: 10.1002/ana.22551
27. Darnell RB, Posner JB. Paraneoplastic syndromes involving the nervous system. *N Engl J Med.* (2003) 349:1543–54. doi: 10.1056/NEJMra023009
28. Gultekin SH, Rosenfeld MR, Voltz R, Eichen J, Posner JB, Dalmau J. Paraneoplastic limbic encephalitis: neurological symptoms, immunological findings and tumour association in 50 patients. *Brain.* (2000) 123 (Pt 7):1481–94. doi: 10.1093/brain/123.7.1481
29. Rojas-Marcos I, Rousseau A, Keime-Guibert F, Reñé R, Cartalat-Carel S, Delattre JY, et al. Spectrum of paraneoplastic neurologic disorders in

women with breast and gynecologic cancer. *Medicine.* (2003) 82:216–23. doi: 10.1097/01.md.0000076004.64510.ce
30. Bataller L, Wade DF, Graus F, Stacey HD, Rosenfeld MR, Dalmau J. Antibodies to Zic4 in paraneoplastic neurologic disorders and small-cell lung cancer. *Neurology.* (2004) 62:778–82. doi: 10.1212/01.WNL.0000113749.77217.01
31. Dalmau J, Tüzün E, Wu HY, Masjuan J, Rossi JE, Voloschin A, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. *Ann Neurol.* (2007) 61:25–36. doi: 10.1002/ana.21050
32. Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R. Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *Lancet Neurol.* (2011) 10:63–74. doi: 10.1016/S1474-4422(10)70253-2
33. Butler MH, Hayashi A, Ohkoshi N, Villmann C, Becker CM, Feng G, et al. Autoimmunity to gephyrin in Stiff-Man syndrome. *Neuron.* (2000) 26:307–12. doi: 10.1016/S0896-6273(00)81165-4
34. Hernández-Echebarría L, Saiz A, Arés A, Tejada J, García-Tuñón L, Nieves C, et al. Paraneoplastic encephalomyelitis associated with pancreatic tumor and anti-GAD antibodies. *Neurology.* (2006) 66:450–1. doi: 10.1212/01.wnl.0000196488.87746.7b
35. Saiz A, Blanco Y, Sabater L, González F, Bataller L, Casamitjana R, et al. Spectrum of neurological syndromes associated with glutamic acid decarboxylase antibodies: diagnostic clues for this association. *Brain.* (2008) 131(Pt 10):2553–63. doi: 10.1093/brain/awn183
36. Shams'ili S, Grefkens J, de Leeuw B, van den Bent M, Hooijkaas H, van der Holt B, et al. Paraneoplastic cerebellar degeneration associated with antineuronal antibodies: analysis of 50 patients. *Brain.* (2003) 126(Pt 6):1409–18. doi: 10.1093/brain/awg133
37. Jernigan M, Morcos Y, Lee SM, Dohan FC, Raine C, Levin MC. IgG in brain correlates with clinicopathological damage in HTLV-1 associated neurologic disease. *Neurology.* (2003) 60:1320–7. doi: 10.1212/01.WNL.0000059866.03880.BA
38. Kalume F, Lee SM, Morcos Y, Callaway JC, Levin MC. Molecular mimicry: cross-reactive antibodies from patients with immune-mediated neurologic disease inhibit neuronal firing. *J Neurosci Res.* (2004) 77:82–9. doi: 10.1002/jnr.20137
39. Amevigbe MD, Jauberteau-Marchan MO, Bouteille B, Doua F, Breton JC, Nicolas JA, et al. Human African trypanosomiasis: presence of antibodies to galactocerebrosides. *Am J Trop Med Hyg.* (1992) 47:652–62. doi: 10.4269/ajtmh.1992.47.652
40. Asonganyi T, Lando G, Ngu JL. Serum antibodies against human brain myelin proteins in Gambian trypanosomiasis. *Ann Soc Belg Med Trop.* (1989) 69:213–21.
41. Hunter CA, Jennings FW, Tierney JF, Murray M, Kennedy PG. Correlation of autoantibody titres with central nervous system pathology in experimental African trypanosomiasis. *J Neuroimmunol.* (1992) 41:143–8. doi: 10.1016/0165-5728(92)90064-R
42. Brimberg L, Benhar I, Mascaro-Blanco A, Alvarez K, Lotan D, Winter C, et al. Behavioral, pharmacological, and immunological abnormalities after streptococcal exposure: a novel rat model of Sydenham chorea and related neuropsychiatric disorders. *Neuropsychopharmacology.* (2012) 37:2076–87. doi: 10.1038/npp.2012.56
43. Garvey MA, Snider LA, Leitman SF, Werden R, Swedo SE. Treatment of Sydenham's chorea with intravenous immunoglobulin, plasma exchange, or prednisone. *J Child Neurol.* (2005) 20:424–9. doi: 10.1177/08830738050200050601
44. Kirvan CA, Swedo SE, Heuser JS, Cunningham MW. Mimicry and autoantibody-mediated neuronal cell signaling in Sydenham chorea. *Nat Med.* (2003) 9:914–20. doi: 10.1038/nm892
45. Kirvan CA, Swedo SE, Kurahara D, Cunningham MW. Streptococcal mimicry and antibody-mediated cell signaling in the pathogenesis of Sydenham's chorea. *Autoimmunity.* (2006) 39:21–9. doi: 10.1080/08916930500484757
46. Kirvan CA, Cox CJ, Swedo SE, Cunningham MW. Tubulin is a neuronal target of autoantibodies in Sydenham's chorea. *J Immunol.* (2007) 178:7412–21. doi: 10.4049/jimmunol.178.11.7412
47. Yaddanapudi K, Hornig M, Serge R, De Miranda J, Baghban A, Villar G, et al. Passive transfer of streptococcus-induced antibodies reproduces behavioral disturbances in a mouse model of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection. *Mol Psychiatry.* (2010) 15:712–26. doi: 10.1038/mp.2009.77
48. Dewing P, Shi T, Horvath S, Vilain E. Sexually dimorphic gene expression in mouse brain precedes gonadal differentiation. *Brain Res Mol Brain Res.* (2003) 118:82–90. doi: 10.1016/S0169-328X(03)00339-5
49. Trabzuni D, Ramasamy A, Imran S, Walker R, Smith C, Weale ME, et al. Widespread sex differences in gene expression and splicing in the adult human brain. *Nat Commun.* (2013) 4:2771. doi: 10.1038/ncomms3771
50. Xu J, Burgoyne PS, Arnold AP. Sex differences in sex chromosome gene expression in mouse brain. *Hum Mol Genet.* (2002) 11:1409–19. doi: 10.1093/hmg/11.12.1409
51. Katzav A, Ben-Ziv T, Chapman J, Blank M, Reichlin M, Shoenfeld Y. Anti-P ribosomal antibodies induce defect in smell capability in a model of CNS -SLE (depression). *J Autoimmun.* (2008) 31:393–8. doi: 10.1016/j.jaut.2008.09.002
52. Katzav A, Solodeev I, Brodsky O, Chapman J, Pick CG, Blank M, et al. Induction of autoimmune depression in mice by anti-ribosomal P antibodies via the limbic system. *Arthritis Rheum.* (2007) 56:938–48. doi: 10.1002/art.22419
53. Gluecksohn-Waelsch S. The effect of maternal immunization against organ tissues on embryonic differentiation in the mouse. *J Embryol Exp Morphol.* (1957) 5:83.
54. Karpiak SE, Rapport MM. Behavioral changes in 2-month-old rats following prenatal exposure to antibodies against synaptic membranes. *Brain Res.* (1975) 92:405–13. doi: 10.1016/0006-8993(75)90325-X
55. McAllister DL, Kaplan BJ, Edworthy SM, Martin L, Crawford SG, Ramsey-Goldman R, et al. The influence of systemic lupus erythematosus on fetal development: cognitive, behavioral, and health trends. *J Int Neuropsychol Soc.* (1997) 3:370–6.
56. Ross G, Sammaritano L, Nass R, Lockshin M. Effects of mothers' autoimmune disease during pregnancy on learning disabilities and hand preference in their children. *Arch Pediatr Adolesc Med.* (2003) 157:397–402. doi: 10.1001/archpedi.157.4.397
57. Urowitz MB, Gladman DD, MacKinnon A, Ibañez D, Bruto V, Rovet J, et al. Neurocognitive abnormalities in offspring of mothers with systemic lupus erythematosus. *Lupus.* (2008) 17:555–60. doi: 10.1177/0961203308089326
58. Dalsgaard S, Waltoft BL, Leckman JF, Mortensen PB. Maternal history of autoimmune disease and later development of tourette syndrome in offspring. *J Am Acad Child Adolesc Psychiatry.* (2015) 54:495–501.e491. doi: 10.1016/j.jaac.2015.03.008
59. Sweeten TL, Bowyer SL, Posey DJ, Halberstadt GM, McDougle CJ. Increased prevalence of familial autoimmunity in probands with pervasive developmental disorders. *Pediatrics.* (2003) 112:e420. doi: 10.1542/peds.112.5.e420
60. Ghassabian A, Bongers-Schokking JJ, de Rijke YB, van Mil N, Jaddoe VW, de Muinck Keizer-Schrama SM, et al. Maternal thyroid autoimmunity during pregnancy and the risk of attention deficit/hyperactivity problems in children: the Generation R Study. *Thyroid.* (2012) 22:178–86. doi: 10.1089/thy.2011.0318
61. Atladóttir HO, Pedersen MG, Thorsen P, Mortensen PB, Deleuran B, Eaton WW, et al. Association of family history of autoimmune diseases and autism spectrum disorders. *Pediatrics.* (2009) 124:687–94. doi: 10.1542/peds.2008-2445
62. Comi AM, Zimmerman AW, Frye VH, Law PA, Peeden JN. Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism. *J Child Neurol.* (1999) 14:388–94. doi: 10.1177/088307389901400608
63. Croen LA, Grether JK, Yoshida CK, Odouli R, Van de Water J. Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. *Arch Pediatr Adolesc Med.* (2005) 159:151–7. doi: 10.1001/archpedi.159.2.151
64. Mostafa GA, Shehab AA. The link of C4B null allele to autism and to a family history of autoimmunity in Egyptian autistic children. *J Neuroimmunol.* (2010) 223:115–9. doi: 10.1016/j.jneuroim.2010.03.025
65. Mostafa GA, El-Sherif DF, Al-Ayadhi LY. Systemic auto-antibodies in children with autism. *J Neuroimmunol.* (2014) 272:94–8. doi: 10.1016/j.jneuroim.2014.04.011
66. Vinet É, Pineau CA, Clarke AE, Scott S, Fombonne É, Joseph L, et al. Increased risk of autism spectrum disorders in children born to women with systemic lupus erythematosus: results from a large population-based cohort. *Arthritis Rheumatol.* (2015) 67:3201–8. doi: 10.1002/art.39320

67. Brimberg L, Sadiq A, Gregersen PK, Diamond B. Brain-reactive IgG correlates with autoimmunity in mothers of a child with an autism spectrum disorder. *Mol Psychiatry.* (2013) 18:1171–7. doi: 10.1038/mp.2013.101
68. Kim J, Mohanty S, Ganesan LP, Hua K, Jarjoura D, Hayton WL, et al. FcRn in the yolk sac endoderm of mouse is required for IgG transport to fetus. *J Immunol.* (2009) 182:2583–9. doi: 10.4049/jimmunol.0803247
69. Malek A, Sager R, Kuhn P, Nicolaides KH, Schneider H. Evolution of maternofetal transport of immunoglobulins during human pregnancy. *Am J Reprod Immunol.* (1996) 36:248–55. doi: 10.1111/j.1600-0897.1996.tb00172.x
70. Palmeira P, Quinello C, Silveira-Lessa AL, Zago CA, Carneiro-Sampaio M. IgG placental transfer in healthy and pathological pregnancies. *Clin Dev Immunol.* (2012) 2012:985646. doi: 10.1155/2012/985646
71. Sarvas H, Seppälä I, Kurikka S, Siegberg R, Mäkelä O. Half-life of the maternal IgG1 allotype in infants. *J Clin Immunol.* (1993) 13:145–51. doi: 10.1007/BF00919271
72. Diamond B, Huerta PT, Mina-Osorio P, Kowal C, Volpe BT. Losing your nerves? Maybe it's the antibodies. *Nat Rev Immunol.* (2009) 9:449–56. doi: 10.1038/nri2529
73. American Psychiatric Association and American Psychiatric Association. DSM-5 Task Force (2013). *Diagnostic and Statistical Manual of Mental Disorders: DSM-5.* 5th ed. Washington, DC: American Psychiatric Association. doi: 10.1176/appi.books.9780890425596
74. Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, et al. Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2014. *MMWR Surveill Summ.* (2018) 67:1–23. doi: 10.15585/mmwr.ss6706a1
75. Bernier R, Golzio C, Xiong B, Stessman HA, Coe BP, Penn O, et al. Disruptive CHD8 mutations define a subtype of autism early in development. *Cell.* (2014) 158:263–76. doi: 10.1016/j.cell.2014.06.017
76. Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, et al. Strong association of *de novo* copy number mutations with autism. *Science.* (2007) 316:445–9. doi: 10.1126/science.1138659
77. Betancur C. Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting. *Brain Res.* (2011) 1380:42–77. doi: 10.1016/j.brainres.2010.11.078
78. Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, et al. Genetic heritability and shared environmental factors among twin pairs with autism. *Arch Gen Psychiatry.* (2011) 68:1095–102. doi: 10.1001/archgenpsychiatry.2011.76
79. Dalton P, Deacon R, Blamire A, Pike M, McKinlay I, Stein J, et al. Maternal neuronal antibodies associated with autism and a language disorder. *Ann Neurol.* (2003) 53:533–7. doi: 10.1002/ana.10557
80. Croen LA, Braunschweig D, Haapanen L, Yoshida CK, Fireman B, Grether JK, et al. Maternal mid-pregnancy autoantibodies to fetal brain protein: the early markers for autism study. *Biol Psychiatry.* (2008) 64:583–8. doi: 10.1016/j.biopsych.2008.05.006
81. Braunschweig D, Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Croen LA, et al. Autism: maternally derived antibodies specific for fetal brain proteins. *Neurotoxicology.* (2008) 29:226–31. doi: 10.1016/j.neuro.2007.10.010
82. Braunschweig D, Golub MS, Koenig CM, Qi L, Pessah IN, Van de Water J, et al. Maternal autism-associated IgG antibodies delay development and produce anxiety in a mouse gestational transfer model. *J Neuroimmunol.* (2012) 252:56–65. doi: 10.1016/j.jneuroim.2012.08.002
83. Braunschweig D, Krakowiak P, Duncanson P, Boyce R, Hansen RL, Ashwood P, et al. Autism-specific maternal autoantibodies recognize critical proteins in developing brain. *Transl Psychiatry.* (2013) 3:e277. doi: 10.1038/tp.2013.50
84. Edmiston E, Jones KL, Vu T, Ashwood P, Van de Water J. Identification of the antigenic epitopes of maternal autoantibodies in autism spectrum disorders. *Brain Behav Immun.* (2018) 69:399–407. doi: 10.1016/j.bbi.2017.12.014
85. Piras IS, Haapanen L, Napolioni V, Sacco R, Van de Water J, Persico AM. Anti-brain antibodies are associated with more severe cognitive and behavioral profiles in Italian children with Autism Spectrum Disorder. *Brain Behav Immun.* (2014) 38:91–9. doi: 10.1016/j.bbi.2013.12.020
86. Rossi CC, Fuentes J, Van de Water J, Amaral DG. Brief report: antibodies reacting to brain tissue in basque spanish children with autism spectrum disorder and their mothers. *J Autism Dev Disord.* (2014) 44:459–465. doi: 10.1007/s10803-013-1859-y
87. Nordahl CW, Braunschweig D, Iosif AM, Lee A, Rogers S, Ashwood P, et al. Maternal autoantibodies are associated with abnormal brain enlargement in a subgroup of children with autism spectrum disorder. *Brain Behav Immun.* (2013) 30:61–5. doi: 10.1016/j.bbi.2013.01.084
88. Singer HS, Morris CM, Gause CD, Gillin PK, Crawford S, Zimmerman AW. Antibodies against fetal brain in sera of mothers with autistic children. *J Neuroimmunol.* (2008) 194:165–72. doi: 10.1016/j.jneuroim.2007.11.004
89. Zimmerman AW, Connors SL, Matteson KJ, Lee LC, Singer HS, Castaneda JA, et al. Maternal antibrain antibodies in autism. *Brain Behav Immun.* (2007) 21:351–7. doi: 10.1016/j.bbi.2006.08.005
90. Ali NH, Khalaf SK, Al-Asadi JN, Abed AH. Maternal antineuronal antibodies and risk of childhood autism spectrum disorders: a case-control study. *J Chin Med Assoc.* (2016) 79:661–4. doi: 10.1016/j.jcma.2016.08.003
91. Brimberg L, Mader S, Jeganathan V, Berlin R, Coleman TR, Gregersen PK, et al. Caspr2-reactive antibody cloned from a mother of an ASD child mediates an ASD-like phenotype in mice. *Mol Psychiatry.* (2016) 21:1663–71. doi: 10.1038/mp.2016.165
92. Martin LA, Ashwood P, Braunschweig D, Cabanlit M, Van de Water J, Amaral DG. Stereotypies and hyperactivity in rhesus monkeys exposed to IgG from mothers of children with autism. *Brain Behav Immun.* (2008) 22:806–16. doi: 10.1016/j.bbi.2007.12.007
93. Braunschweig D, Duncanson P, Boyce R, Hansen R, Ashwood P, Pessah IN, et al. Behavioral correlates of maternal antibody status among children with autism. *J Autism Dev Disord.* (2012) 42:1435–45. doi: 10.1007/s10803-011-1378-7
94. Bauman MD, Iosif AM, Ashwood P, Braunschweig D, Lee A, Schumann CM, et al. Maternal antibodies from mothers of children with autism alter brain growth and social behavior development in the rhesus monkey. *Transl Psychiatry.* (2013) 3:e278. doi: 10.1038/tp.2013.47
95. Camacho J, Jones K, Miller E, Ariza J, Noctor S, Van de Water J, et al. Embryonic intraventricular exposure to autism-specific maternal autoantibodies produces alterations in autistic-like stereotypical behaviors in offspring mice. *Behav Brain Res.* (2014) 266:46–51. doi: 10.1016/j.bbr.2014.02.045
96. Martínez-Cerdeño V, Camacho J, Fox E, Miller E, Ariza J, Kienzle D, et al. Prenatal exposure to autism-specific maternal autoantibodies alters proliferation of cortical neural precursor cells, enlarges brain, and increases neuronal size in adult animals. *Cereb Cortex.* (2016) 26:374–83. doi: 10.1093/cercor/bhu291
97. Ariza J, Hurtado J, Rogers H, Ikeda R, Dill M, Steward C, et al. Maternal autoimmune antibodies alter the dendritic arbor and spine numbers in the infragranular layers of the cortex. *PLoS ONE.* (2017) 12:e0183443. doi: 10.1371/journal.pone.0183443
98. Jones KL, Pride MC, Edmiston E, Yang M, Silverman JL, Crawley JN, et al. Autism-specific maternal autoantibodies produce behavioral abnormalities in an endogenous antigen-driven mouse model of autism. *Mol Psychiatry.* (2018). doi: 10.1038/s41380-018-0126-1. [Epub ahead of print]
99. Singer HS, Morris C, Gause C, Pollard M, Zimmerman AW, Pletnikov M. Prenatal exposure to antibodies from mothers of children with autism produces neurobehavioral alterations: a pregnant dam mouse model. *J Neuroimmunol.* (2009) 211:39–48. doi: 10.1016/j.jneuroim.2009.03.011
100. Kadam SD, French BM, Kim ST, Morris-Berry CM, Zimmerman AW, Blue ME, et al. Altered postnatal cell proliferation in brains of mouse pups prenatally exposed to IgG from mothers of children with autistic disorder. *J Exp Neurosci.* (2013) 7:93–9. doi: 10.4137/JEN.S12979
101. Lee JY, Huerta PT, Zhang J, Kowal C, Bertini E, Volpe BT, et al. Neurotoxic autoantibodies mediate congenital cortical impairment of offspring in maternal lupus. *Nat Med.* (2009) 15:91–6. doi: 10.1038/nm.1892
102. Wang L, Zhou D, Lee J, Niu H, Faust TW, Frattini S, et al. Female mouse fetal loss mediated by maternal autoantibody. *J Exp Med.* (2012) 209:1083–9. doi: 10.1084/jem.20111986
103. Bel C, Oguievetskaia K, Pitaval C, Goutebroze L, Faivre-Sarrailh C. Axonal targeting of Caspr2 in hippocampal neurons via selective somatodendritic endocytosis. *J Cell Sci.* (2009) 122(Pt 18):3403–13. doi: 10.1242/jcs.050526
104. Poliak S, Gollan L, Martinez R, Custer A, Einheber S, Salzer JL, et al. Caspr2, a new member of the neurexin superfamily, is localized at the juxtaparanodes of myelinated axons and associates with K+ channels. *Neuron.* (1999) 24:1037–47. doi: 10.1016/S0896-6273(00)81049-1

105. Alarcón M, Abrahams BS, Stone JL, Duvall JA, Perederiy JV, Bomar JM, et al. Linkage, association, and gene-expression analyses identify CNTNAP2 as an autism-susceptibility gene. *Am J Hum Genet.* (2008) 82:150–9. doi: 10.1016/j.ajhg.2007.09.005
106. Arking DE, Cutler DJ, Brune CW, Teslovich TM, West K, Ikeda M, et al. A common genetic variant in the neurexin superfamily member CNTNAP2 increases familial risk of autism. *Am J Hum Genet.* (2008) 82:160–4. doi: 10.1016/j.ajhg.2007.09.015
107. Bakkaloglu B, O'Roak BJ, Louvi A, Gupta AR, Abelson JF, Morgan TM, et al. Molecular cytogenetic analysis and resequencing of contactin associated protein-like 2 in autism spectrum disorders. *Am J Hum Genet.* (2008) 82:165–73. doi: 10.1016/j.ajhg.2007.09.017
108. Newbury DF, Paracchini S, Scerri TS, Winchester L, Addis L, Richardson AJ, et al. Investigation of dyslexia and SLI risk variants in reading- and language-impaired subjects. *Behav Genet.* (2011) 41:90–104. doi: 10.1007/s10519-010-9424-3
109. Nord AS, Roeb W, Dickel DE, Walsh T, Kusenda M, O'Connor KL, et al. Reduced transcript expression of genes affected by inherited and *de novo* CNVs in autism. *Eur J Hum Genet.* (2011) 19:727–31. doi: 10.1038/ejhg.2011.24
110. O'Roak BJ, Deriziotis P, Lee C, Vives L, Schwartz JJ, Girirajan S, et al. Exome sequencing in sporadic autism spectrum disorders identifies severe *de novo* mutations. *Nat Genet.* (2011) 43:585–9. doi: 10.1038/ng.835
111. Poot M, Beyer V, Schwaab I, Damatova N, Van't Slot R, Prothero J, et al. Disruption of CNTNAP2 and additional structural genome changes in a boy with speech delay and autism spectrum disorder. *Neurogenetics.* (2010) 11:81–9. doi: 10.1007/s10048-009-0205-1
112. Sehested LT, Møller RS, Bache I, Andersen NB, Ullmann R, Tommerup N, et al. Deletion of 7q34-q36.2 in two siblings with mental retardation, language delay, primary amenorrhea, and dysmorphic features. *Am J Med Genet A.* (2010) 152A:3115–9. doi: 10.1002/ajmg.a.33476
113. Steer CD, Golding J, Bolton PF. Traits contributing to the autistic spectrum. *PLoS ONE.* (2010) 5:e12633. doi: 10.1371/journal.pone.0012633
114. Strauss KA, Puffenberger EG, Huentelman MJ, Gottlieb S, Dobrin SE, Parod JM, et al. Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. *N Engl J Med.* (2006) 354:1370–7. doi: 10.1056/NEJMoa052773
115. Vernes SC, Newbury DF, Abrahams BS, Winchester L, Nicod J, Groszer M, et al. A functional genetic link between distinct developmental language disorders. *N Engl J Med.* (2008) 359:2337–45. doi: 10.1056/NEJMoa0802828
116. Peñagarikano O, Abrahams BS, Herman EI, Winden KD, Gdalyahu A, Dong H, et al. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. *Cell.* (2011) 147:235–46. doi: 10.1016/j.cell.2011.08.040
117. Baron-Cohen S, Lombardo MV, Auyeung B, Ashwin E, Chakrabarti B, Knickmeyer R. Why are autism spectrum conditions more prevalent in males? *PLoS Biol.* (2011) 9:e1001081. doi: 10.1371/journal.pbio.1001081
118. Nolen-Hoeksema S, Girgus JS. The emergence of gender differences in depression during adolescence. *Psychol Bull.* (1994) 115:424–43. doi: 10.1037/0033-2909.115.3.424
119. Rutter M, Caspi A, Moffitt TE. Using sex differences in psychopathology to study causal mechanisms: unifying issues and research strategies. *J Child Psychol Psychiatry.* (2003) 44:1092–115. doi: 10.1111/1469-7610.00194
120. Dewing P, Chiang CW, Sinchak K, Sim H, Fernagut PO, Kelly S, et al. Direct regulation of adult brain function by the male-specific factor SRY. *Curr Biol.* (2006) 16:415–20. doi: 10.1016/j.cub.2006.01.017
121. De Vries GJ, Rissman EF, Simerly RB, Yang LY, Scordalakes EM, Auger CJ, et al. A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits. *J Neurosci.* (2002) 22:9005–14. doi: 10.1523/JNEUROSCI.22-20-09005.2002
122. Carruth LL, Reisert I, Arnold AP. Sex chromosome genes directly affect brain sexual differentiation. *Nat Neurosci.* (2002) 5:933–4. doi: 10.1038/nn922
123. Cox KH, Rissman EF. Sex differences in juvenile mouse social behavior are influenced by sex chromosomes and social context. *Genes Brain Behav.* (2011) 10:465–72. doi: 10.1111/j.1601-183X.2011.00688.x
124. McPhie-Lalmansingh AA, Tejada LD, Weaver JL, Rissman EF. Sex chromosome complement affects social interactions in mice. *Horm Behav.* (2008) 54:565–70. doi: 10.1016/j.yhbeh.2008.05.016
125. Gatewood JD, Wills A, Shetty S, Xu J, Arnold AP, Burgoyne PS, et al. Sex chromosome complement and gonadal sex influence aggressive and parental behaviors in mice. *J Neurosci.* (2006) 26:2335–42. doi: 10.1523/JNEUROSCI.3743-05.2006
126. Maxson SC, Didier-Erickson A, Ogawa S. The Y chromosome, social signals, and offense in mice. *Behav Neural Biol.* (1989) 52:251–9. doi: 10.1016/S0163-1047(89)90369-5
127. Sluyter F, Bohus B, Beldhuis HJ, van Oortmerssen GA. Autosomal and Y chromosomal effects on the stereotyped response to apomorphine in wild house mice. *Pharmacol Biochem Behav.* (1995) 52:17–22. doi: 10.1016/0091-3057(95)00092-B
128. Kopsida E, Lynn PM, Humby T, Wilkinson LS, Davies W. Dissociable effects of Sry and sex chromosome complement on activity, feeding and anxiety-related behaviours in mice. *PLoS ONE.* (2013) 8:e73699. doi: 10.1371/journal.pone.0073699
129. Carrel L, Cottle AA, Goglin KC, Willard HF. A first-generation X-inactivation profile of the human X chromosome. *Proc Natl Acad Sci USA.* (1999) 96:14440–4. doi: 10.1073/pnas.96.25.14440
130. Carrel L, Willard HF. X-inactivation profile reveals extensive variability in X-linked gene expression in females. *Nature.* (2005) 434:400–4. doi: 10.1038/nature03479
131. Yang F, Babak T, Shendure J, Disteche CM. Global survey of escape from X inactivation by RNA-sequencing in mouse. *Genome Res.* (2010) 20:614–22. doi: 10.1101/gr.103200.109
132. Gregg C, Zhang J, Butler JE, Haig D, Dulac C. Sex-specific parent-of-origin allelic expression in the mouse brain. *Science.* (2010) 329:682–5. doi: 10.1126/science.1190831
133. Raefski AS, O'Neill MJ. Identification of a cluster of X-linked imprinted genes in mice. *Nat Genet.* (2005) 37:620–4. doi: 10.1038/ng1567
134. Skuse DH, James RS, Bishop DV, Coppin B, Dalton P, Aamodt-Leeper G, et al. Evidence from Turner's syndrome of an imprinted X-linked locus affecting cognitive function. *Nature.* (1997) 387:705–8. doi: 10.1038/42706
135. Arai Y, Sekine Y, Murakami S. Estrogen and apoptosis in the developing sexually dimorphic preoptic area in female rats. *Neurosci Res.* (1996) 25:403–7. doi: 10.1016/0168-0102(96)01070-X
136. Arnold AP, Gorski RA. Gonadal steroid induction of structural sex differences in the central nervous system. *Annu Rev Neurosci.* (1984) 7:413–42. doi: 10.1146/annurev.ne.07.030184.002213
137. Brinton RD, Tran J, Proffitt P, Montoya M. 17 beta-Estradiol enhances the outgrowth and survival of neocortical neurons in culture. *Neurochem Res.* (1997) 22:1339–51. doi: 10.1023/A:1022015005508
138. Gould E, Woolley CS, Frankfurt M, McEwen BS. Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J Neurosci.* (1990) 10:1286–91. doi: 10.1523/JNEUROSCI.10-04-01286.1990
139. Nilsen J, Mor G, Naftolin F. Estrogen-regulated developmental neuronal apoptosis is determined by estrogen receptor subtype and the Fas/Fas ligand system. *J Neurobiol.* (2000) 43:64–78. doi: 10.1002/(SICI)1097-4695(200004)43:1<64::AID-NEU6>3.0.CO;2-7
140. Wang L, Andersson S, Warner M, Gustafsson JA. Estrogen receptor (ER)beta knockout mice reveal a role for ERbeta in migration of cortical neurons in the developing brain. *Proc Natl Acad Sci USA.* (2003) 100:703–8. doi: 10.1073/pnas.242735799
141. Wu MV, Manoli DS, Fraser EJ, Coats JK, Tollkuhn J, Honda S, et al. Estrogen masculinizes neural pathways and sex-specific behaviors. *Cell.* (2009) 139:61–72. doi: 10.1016/j.cell.2009.07.036
142. Wu MV, Shah NM. Control of masculinization of the brain and behavior. *Curr Opin Neurobiol.* (2011) 21:116–23. doi: 10.1016/j.conb.2010.09.014
143. Knickmeyer R, Baron-Cohen S, Raggatt P, Taylor K. Foetal testosterone, social relationships, and restricted interests in children. *J Child Psychol Psychiatry.* (2005) 46:198–210. doi: 10.1111/j.1469-7610.2004.00349.x
144. Knickmeyer R, Baron-Cohen S, Raggatt P, Taylor K, Hackett G. Fetal testosterone and empathy. *Horm Behav.* (2006) 49:282–92. doi: 10.1016/j.yhbeh.2005.08.010
145. Lutchmaya S, Baron-Cohen S, Raggatt P. Foetal testosterone and eye contact in 12-month-old human infants. *Infant Behav Dev.* (2002) 25:327–35. doi: 10.1016/S0163-6383(02)00094-2
146. Hoffman EJ, Turner KJ, Fernandez JM, Cifuentes D, Ghosh M, Ijaz S, et al. Estrogens suppress a behavioral phenotype in zebrafish mutants of the autism risk gene, CNTNAP2. *Neuron.* (2016) 89:725–33. doi: 10.1016/j.neuron.2015.12.039

147. Macrì S, Biamonte F, Romano E, Marino R, Keller F, Laviola G. Perseverative responding and neuroanatomical alterations in adult heterozygous reeler mice are mitigated by neonatal estrogen administration. *Psychoneuroendocrinology.* (2010) 35:1374–87. doi: 10.1016/j.psyneuen.2010.03.012
148. vom Saal FS. Sexual differentiation in litter-bearing mammals: influence of sex of adjacent fetuses *in utero*. *J Anim Sci.* (1989) 67:1824–40. doi: 10.2527/jas1989.6771824x
149. Itoh Y, Mackie R, Kampf K, Domadia S, Brown JD, O'Neill R, et al. Four core genotypes mouse model: localization of the Sry transgene and bioassay for testicular hormone levels. *BMC Res Notes.* (2015) 8:69. doi: 10.1186/s13104-015-0986-2
150. Smith-Bouvier DL, Divekar AA, Sasidhar M, Du S, Tiwari-Woodruff SK, King JK, et al. A role for sex chromosome complement in the female bias in autoimmune disease. *J Exp Med.* (2008) 205:1099–108. doi: 10.1084/jem.20070850
151. D'Eufemia P, Celli M, Finocchiaro R, Pacifico L, Viozzi L, Zaccagnini M, et al. Abnormal intestinal permeability in children with autism. *Acta Paediatr.* (1996) 85:1076–9. doi: 10.1111/j.1651-2227.1996.tb14220.x
152. de Magistris L, Familiari V, Pascotto A, Sapone A, Frolli A, Iardino P, et al. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. *J Pediatr Gastroenterol Nutr.* (2010) 51:418–24. doi: 10.1097/MPG.0b013e3181dcc4a5
153. Kohane IS, McMurry A, Weber G, MacFadden D, Rappaport L, Kunkel L, et al. The co-morbidity burden of children and young adults with autism spectrum disorders. *PLoS ONE.* (2012) 7:e33224. doi: 10.1371/journal.pone.0033224
154. Buie T, Campbell DB, Fuchs GJ, Furuta GT, Levy J, Vandewater J, et al. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. *Pediatrics.* (2010) 125(Suppl. 1):S1–18. doi: 10.1542/peds.2009-1878C
155. Coury DL, Ashwood P, Fasano A, Fuchs G, Geraghty M, Kaul A, et al. Gastrointestinal conditions in children with autism spectrum disorder: developing a research agenda. *Pediatrics.* (2012) 130(Suppl. 2):S160–8. doi: 10.1542/peds.2012-0900N
156. Adams JB, Johansen LJ, Powell LD, Quig D, Rubin RA. Gastrointestinal flora and gastrointestinal status in children with autism–comparisons to typical children and correlation with autism severity. *BMC Gastroenterol.* (2011) 11:22. doi: 10.1186/1471-230X-11-22
157. McElhanon BO, McCracken C, Karpen S, Sharp WG. Gastrointestinal symptoms in autism spectrum disorder: a meta-analysis. *Pediatrics.* (2014) 133:872–83. doi: 10.1542/peds.2013-3995
158. Arentsen T, Raith H, Qian Y, Forssberg H, Diaz Heijtz R. Host microbiota modulates development of social preference in mice. *Microb Ecol Health Dis.* (2015) 26:29719. doi: 10.3402/mehd.v26.29719
159. Bercik P, Park AJ, Sinclair D, Khoshdel A, Lu J, Huang X, et al. The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterol Motil.* (2011) 23:1132–9. doi: 10.1111/j.1365-2982.2011.01796.x
160. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci USA.* (2011) 108:16050–5. doi: 10.1073/pnas.1102999108
161. Desbonnet L, Clarke G, Shanahan F, Dinan TG, Cryan JF. Microbiota is essential for social development in the mouse. *Mol Psychiatry.* (2014) 19:146–8. doi: 10.1038/mp.2013.65
162. Desbonnet L, Clarke G, Traplin A, O'Sullivan O, Crispie F, Moloney RD, et al. Gut microbiota depletion from early adolescence in mice: implications for brain and behaviour. *Brain Behav Immun.* (2015) 48:165–73. doi: 10.1016/j.bbi.2015.04.004
163. Diaz Heijtz R, Wang S, Anuar F, Qian Y, Björkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci USA.* (2011) 108:3047–52. doi: 10.1073/pnas.1010529108
164. Neufeld KM, Kang N, Bienenstock J, Foster JA. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol Motil.* (2011) 23:255–64, e119. doi: 10.1111/j.1365-2982.2010.01620.x
165. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci.* (2012) 13:701–12. doi: 10.1038/nrn3346
166. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. *Gastroenterology.* (2011) 141:599–609, 609.e591–3. doi: 10.1053/j.gastro.2011.04.052
167. Clarke G, Grenham S, Scully P, Fitzgerald P, Moloney RD, Shanahan F, et al. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol Psychiatry.* (2013) 18:666–73. doi: 10.1038/mp.2012.77
168. Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, et al. Pyrosequencing study of fecal microflora of autistic and control children. *Anaerobe.* (2010) 16:444–53. doi: 10.1016/j.anaerobe.2010.06.008
169. Finegold SM, Downes J, Summanen PH. Microbiology of regressive autism. *Anaerobe.* (2012) 18:260–2. doi: 10.1016/j.anaerobe.2011.12.018
170. Kang DW, Park JG, Ilhan ZE, Wallstrom G, Labaer J, Adams JB, et al. Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. *PLoS ONE.* (2013) 8:e68322. doi: 10.1371/journal.pone.0068322
171. Parracho HM, Bingham MO, Gibson GR, McCartney AL. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *J Med Microbiol.* (2005) 54(Pt 10):987–91. doi: 10.1099/jmm.0.46101-0
172. Williams BL, Hornig M, Buie T, Bauman ML, Cho Paik M, Wick I, et al. Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. *PLoS ONE.* (2011) 6:e24585. doi: 10.1371/journal.pone.0024585
173. Williams BL, Hornig M, Parekh T, Lipkin WI. Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of Sutterella species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. *MBio.* (2012) 3:e00261-11. doi: 10.1128/mBio.00261-11
174. Kim S, Kim H, Yim YS, Ha S, Atarashi K, Tan TG, et al. Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. *Nature.* (2017) 549:528–32. doi: 10.1038/nature23910
175. Choi GB, Yim YS, Wong H, Kim S, Kim H, Kim SV, et al. The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science.* (2016) 351:933–9. doi: 10.1126/science.aad0314
176. Lammert CR, Frost EL, Bolte AC, Paysour MJ, Shaw ME, Bellinger CE, et al. Cutting edge: critical roles for microbiota-mediated regulation of the immune system in a prenatal immune activation model of autism. *J Immunol.* (2018) 201:845–50. doi: 10.4049/jimmunol.1701755
177. Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell.* (2013) 155:1451–63. doi: 10.1016/j.cell.2013.11.024
178. Boyle CA, Boulet S, Schieve LA, Cohen RA, Blumberg SJ, Yeargin-Allsopp M, et al. Trends in the prevalence of developmental disabilities in US children, 1997-2008. *Pediatrics.* (2011) 127:1034–42. doi: 10.1542/peds.2010-2989

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Semi-quantitative FDG-PET Analysis Increases the Sensitivity Compared With Visual Analysis in the Diagnosis of Autoimmune Encephalitis

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Objective: The purpose of this study is to evaluate the potential diagnostic benefit of SPM-based semi-quantitative FDG-PET analysis in autoimmune encephalitis (AE) compared with visual analysis by experienced neuroradiologists using a larger sample size.

Methods: This observational retrospective case series study was conducted from a tertiary epilepsy center between May 2014 and March 2017. Healthy individuals without any neurologic or psychiatric diseases were recruited as control. We determined brain FDG-PET abnormal glucose metabolism on medial temporal lobe and basal ganglia using semi-quantitative analysis and compared this method with visual analysis at the same time among patients with autoantibody positive AE.

Results: Twenty-eight patients with clinically diagnosed AE and 53 healthy individuals without any neurologic or psychiatric diseases were recruited. On the medial temporal lobe and the basal ganglia, semi-quantitative analysis showed consistency with the visual assessment for whom they had abnormal metabolism by visual assessment. More importantly, 56% patients on medial temporal lobe and 73% patients on the basal ganglia respectively who were not identified by visual inspection can be detected by semi-quantitative analysis, demonstrating the greater sensitivity of semi-quantitative analysis compared with visual assessment.

Significance: This study showed semi-quantitative brain FDG-PET analysis was better than visual analysis in view of observing the abnormal glucose metabolism of patients with autoantibody positive AE. Semi-quantitative FDG-PET analysis appears to be a helpful tool in early diagnosis of patients with AE.

Keywords: autoimmune encephalitis, FDG-PET, semi-quantitative analysis, visual analysis, sensitivity

INTRODUCTION

The clinical diagnosis of autoimmune encephalitis (AE) can be difficult since patients manifest various unspecific symptoms that overlap with other encephalitis such as infectious encephalitis (1). The clinical symptoms are varied and include seizures, rapid cognitive decline, behavioral problems, and so on. The diagnosis can be made according to clinical manifestation and serum and/or cerebrospinal fluid (CSF) autoantibody test. Autoantibody testing is not readily available at many institutions for technical reasons. Even if it is available at some institutions, it will take several weeks to obtain (1). In addition, failure to detect a neural antibody cannot exclude AE when other clinical clues exist (2, 3). The early diagnosis of AE is very important because early immunotherapy may slow, halt, or even reverse the disease process (2, 4, 5), whereas delaying initiation of immunosuppressive therapy may result in serious disability, or even death if left untreated (6). Depending on autoantibody testing to make the diagnosis may significantly delay treatment initiation. Therefore, early diagnosis should not be delayed because of awaiting for serological or CSF autoantibody result (1). Early neuroimaging, as well as the initial neurological assessment, plays a key role in the early diagnosis of AE (1, 7).

Recently, leading authorities on the diagnosis and treatment of AE convened and recommended criteria for the diagnoses of possible, probable and definite AE to mitigate the delay in initiation of therapy while awaiting autoantibody assay results (1). Francesc Graus et al. proposed the diagnosis of AE was made according to the neurological evaluation and the corresponding diagnostic correlated examinations including magnetic resonance imaging (MRI), CSF and serum sampling, as well as electroencephalography (EEG) results (1, 8). Prompt diagnosis often relies on neuroimaging. In terms of brain imaging, the recommended diagnostic framework frequently only depends on cranial MRI. Nevertheless, a relevant proportion of AE patients had normal or non-specific MRI results (8, 9). Therefore, the normal MRI finding does not exclude an immune-mediated process. In AE, 2-deoxy-2-18F fluoro-D-glucose-(18F-FDG) positron emission tomography/computed tomography (PET/CT) imaging has been reported to representatively show hypermetabolism on the medial temporal lobe in MRI-negative patients, suggesting that it is better than MRI in the diagnostic framework (8–10).

Traditionally, whole body 18F-FDG-PET has been utilized to assess for occult malignancy as a cause for AE. Recent publications have begun to explore the added value of brain 18F-FDG PET/CT in evaluating these patients (11–13). Several groups have suggested that 18F-FDG PET/CT may be used to evaluate efficacy of therapy or monitor the suspected disease recurrence (11, 14). FDG-PET appears to be a helpful tool in early diagnosis of patients with AE, especially those with normal MRI scans. Some researchers have tried to use 18F-FDG PET/CT to perform early diagnosis of suspicious AE before acquiring the autoantibody results (8). However, the further studies are needed to verify its predictive value for the early diagnosis of AE, and the larger validation studies are needed (15). Therefore, we would like to ulteriorly find the major reliable evidence on the underlying role of 18F-FDG PET for the earlier diagnosis of AE.

TABLE 1 | Demographics of participants.

Group	Age (years)		Gender	
	Range	Mean ± SD	Male	Female
AE patients ($n = 28$)	34∼78	56.32 ± 10.93	22	6
Healthy individuals ($n = 53$)	59∼69	52.47 ± 6.66	31	22

Despite visual analysis is often the first step of brain 18F-FDG PET reading, the standardization in view of reading images and reporting results is defective now (16). In clinical practice, the lack of expertise and objective semi-quantitative detection methods will hold back the comprehension of the real disease pattern and lead to misinterpreted report. Some automated approaches to analyze 18F-FDG PET data are not applicable for finding the hypermetabolism in AE, since they were developed to diagnose the hypometabolic patterns in Alzheimer's disease (17). In contrast, some SPM-based semi-quantitative measures might protrude the areas of relative hypermetabolism owing to the bias introduced by intensity normalization procedures (18). Moreover, one recent report showed that semi-quantitative analysis can reveal subtle changes, suggesting that semi-quantitative analysis was better than qualitative reporting (19).

In the present study, we modified the semi-quantitative FDG-PET analysis as a biomarker for definite, autoantibody positive AE, allowing for additional region of interest (ROI) analyses of the mesial temporal lobe and basal ganglia. We used this analysis method in patients with antibody positive AE. The aim was to evaluate the potential diagnostic value of this analysis method compared with visual analysis by experienced neuroradiologists.

SUBJECTS AND METHODS

Participants

We recruited 28 AE patients with autoantibody (56 ± 11 years; 22 males) in our Tertiary Epilepsy Center from May 2014 to April 2017. All patients who had undergone cerebral FDG-PET were included in this retrospective study. Fifty-three healthy individuals (52 ± 7 years; 31 males) without any neurologic or psychiatric diseases were recruited. The age of these two group of participants were well matched and showed no significant difference [$t_{(79)} = 1.97$; $p > 0.05$). The detailed demographics of participants were presented in **Table 1**. Written Informed consent to participate the study and for publication for clinical details were obtained from each subject enrolled. The study was approved by the Medical Ethics Committee of Beijing Tiantan Hospital, Capital Medical University and was carried out in accordance with the Declaration of Helsinki.

Cases of AE included in the study were patients presenting with new onset electrographic seizure activity, plus at least two of the following: (1) CSF findings consistent with inflammation [elevated CSF protein >45 mg/dl and/or lymphocytic pleocytosis; elevated CSF immunoglobulin G (IgG) index and/or positive oligoclonal bands (OB)]; (2)

brain MRI or FDG-PET showing signal changes consistent with limbic encephalitis; (3) autoimmune/paraneoplastic antibodies in serum and/or CSF which have been associated with autoimmune encephalitis in previous studies (any neuronal nuclear/cytoplasmic antibody such as anti-Hu, Yo, Ri, Ma2/Ta, CV2/CRMP5, amphiphysin; any neuronal membrane antibody including anti-NMDA-R, CASPR2, AMPA1-R, AMPA2-R, LGI1, and $GABA_B$-R antibody), (4) new onset seizure responding to immunomodulatory therapies. Cases were excluded if there was evidence of another identified cause of the patient's seizures: (1) presence of CSF viral/bacterial/fungal antigens or antibodies or DNA PCR which could explain underlying acute inflammatory brain parenchymal changes, (2) presence of metabolic abnormalities which could have precipitated seizures (severe renal or hepatic failure, malignant hypertension, severe hypo/hyperglycemia), (3) presence of brain structural lesions such as stroke, tumor, traumatic lesions, heterotopias, vascular malformation, abscess or infectious lesion which could have precipitated the presenting seizures.

Neuronal Antibody Measurement

AE in the present study were definitely diagnosed by autoantibody assay. All suspected AE patients underwent serum and CSF antibody test. Serum and CSF samples had been sent for antibody test to the laboratory of neurological immunology of Peking Union Medical College Hospital. Serum and CSF titers for onconeural antibodies anti-Hu, Yo, Ri, CV2/CRMP5, amphiphysin, Ma2/Ta, and the neuronal surface antibodies anti-NMDA-R, CASPR2, AMPA1-R, AMPA2-R, LGI1, and $GABA_B$-R were measured with both cell-based assay and immunohistochemistry in serum and CSF.

Cerebral Imaging Acquisition

The brain ^{18}F-FDG PET/CT scan was performed to evaluate the glucose metabolism of each participant. All participants were fasted for at least 6 h and their blood glucose levels were confirmed to be within the normal range before injection of ^{18}F-FDG. The subjects were injected with 0.10–0.15 mCi/kg of ^{18}F-FDG. Then, after 30 min rest in a dimly lit room, they underwent the brain PET/CT scans (eyes open, reduced ambient noise). PET/CT images were acquired with the use of a multidetector helical PET/CT scanner (Discovery 690, GE Medical Systems). All cerebral FDG-PET studies were done in conjunction with whole-body PET scans (in search of malignancies; brain scan first).

Analysis

Visual Assessment

Previous study demonstrated that AE were usually associated with the abnormalities of glucose metabolism in some brain regions, such as medial temporal lobe and basal ganglia (1). Thus, to assess the glucose metabolism in these brain areas, the PET images of each patient were visually examined by three reviewers independently. One of them was an attending doctor of nuclear medicine (X-B Zhao) with vast experience of reading PET/CT (>10 years) and the other two were experienced neurology specialists [R-J Lv (9 years) and X-Q Shao (15 years)]. Three reviewers were asked to carefully diagnose whether the medial temporal lobe and basal ganglia showed abnormal glucose metabolism or not. In addition, they would assess the lateralization of the abnormality when it existed. All reviewers were blinded from clinical diagnosis of the conditions of either cases or controls. These three specialists discussed together to reach a consensus when their original evaluations were discordant. The Kappa coefficient of three specialists was 0.86.

Semi-quantitative Analysis

Image preprocess

The brain PET scans were also assessed through semi-quantitative analysis. The analysis including preprocessing and statistical analysis, were mainly implemented using the MATLAB and Statistical Parametric Mapping software (SPM12, Wellcome Trust Center for Neuroimaging, London, UK; https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). During the preprocessing, the co-registration between PET metabolism images and CT structural images was firstly performed. Then, the CT images with high resolution were spatial normalized into Montreal Neurological Institute template (MNI). Thus, the PET images were also normalized into this standard templates using the computed space transformation for CT normalization. Finally, the PET scans were resampled to $2 \times 2 \times 2$ mm^3 voxels and spatially smoothed using an isotropic 6 mm full-width-half-maximal (FWHM). Additionally, to remove the bias of global metabolism, each voxel's intensity of PET scans was normalized by dividing the average of the voxels within the highest 20% of intensity (20).

Statistical analysis

In line with the above visual assessment, we also investigated the metabolism of brain regions located in medial temporal lobe and basal ganglia using statistical analysis. Considering the size bias of the abnormalities in these areas, the present study not only examined the entire medial temporal lobe and basal ganglia, but also examined their fine-grained divisions. Using the Human Brainnetome Atlas (21), we defined the following regions of interest (ROIs) within medial temporal lobe: (1) bilateral medial amygdala, (2) bilateral lateral amygdala, (3) bilateral caudal hippocampus, (4) bilateral rostral hippocampus, and (5) entire bilateral medial temporal lobe consisting of 1–4; within basal ganglia: (6) bilateral dorsolateral caudate nucleus, (7) bilateral ventral caudate nucleus, (8) bilateral dorsolateral putamen, (9) bilateral ventromedial putamen, (10) bilateral pallidum, (11) bilateral nucleus accumbens, and (12) entire bilateral basal ganglia consisting of 6–11. The locations of these ROIs in MNI template showed in **Figure 1**.

For each ROI within medial temporal lobe, the z-score was calculated as an indicator of abnormalities of glucose metabolism of each patient deviated from that of the control healthy group. Specifically, we firstly calculated the mean of whole voxels' intensities within each ROI to obtain the average metabolic of the ROI for each participant. Then, for each ROI, we calculated the mean (M) and standard deviation (SD) of the average metabolic cross all healthy individuals. Finally, for each patient, z-score was obtained by subtracting M from each ROI and

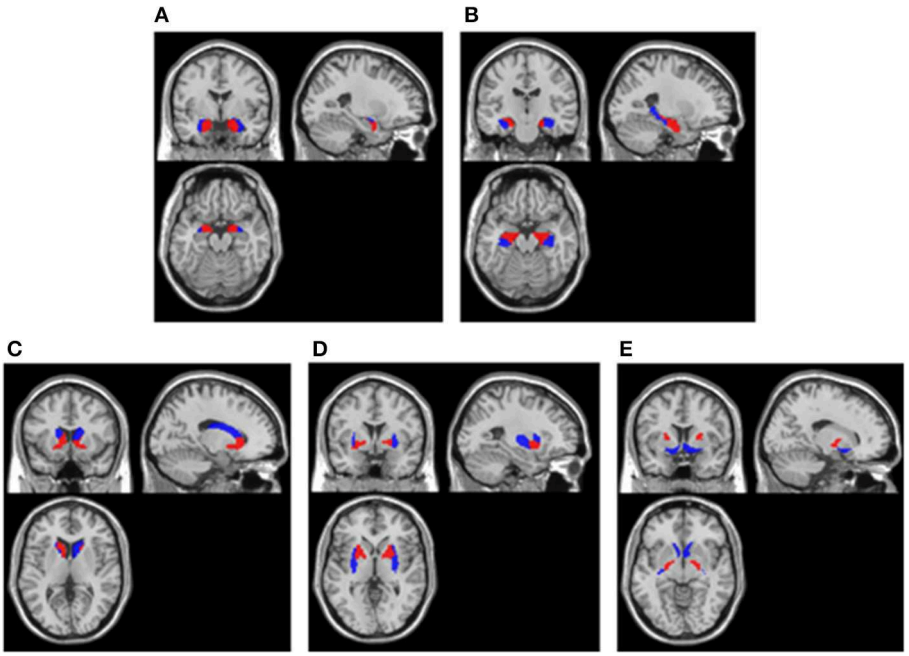

FIGURE 1 | ROIs extracted in cerebral cortex. **(A)** Amygdala. Blue areas were lateral amygdala and red areas were medial amygdala; **(B)** Hippocampus. Blue areas were caudal hippocampus and red areas were rostral hippocampus; **(C)** Caudate nucleus. Blue areas were dorsolateral caudate nucleus and red areas were ventral caudate nucleus; **(D)** Putamen. Blue areas were dorsolateral putamen and red areas were ventromedial putamen; **(E)** Pallidum and Nucleus accumbens. Blue areas were nucleus accumbens and red areas were pallidum. All ROIs were extracted using the Human Brainnetome Atlas (21) and they were overlay on a MNI single subject T1 scan ("/spm12/canonical/single_subj_T1.nii").

then dividing the SD [namely, z-score = (patient-M healthy subjects)/SD healthy subjects]. The medial temporal lobe were identified hyper-metabolism if any of ROIs within the medial temporal lobe showed a significant higher metabolism than healthy group ($z > 1.96$, $p < 0.05$). Whereas, the medial temporal lobe were identified hypo-metabolism if any of ROIs within the medial temporal lobe showed a significant lower metabolism than healthy group ($z < -1.96$, $p < 0.05$). For each ROI within basal ganglia, the same statistical analysis as the ROIs within medial temporal lobe was conducted on each patient.

RESULTS

Autoantibody Assay

The outcome of autoimmune antibody subtypes of each patient was showed in **Table 2**. Twenty-three patients were diagnosed as anti-LGI1 encephalitis, and four patients were diagnosed as anti-$GABA_BR$ encephalitis, and the other one was diagnosed as anti-amphiphysin encephalitis.

Visual Assessment Results

The visual assessment results were shown in **Table 2**. On medial temporal lobe, 19 patients were verified as hyper-metabolism. However, the visual assessment failed to identify the other nine patients. Thus, the sensitivity of visual assessment for autoimmune encephalitis on medial temporal lobe was about 68% (19/28). One patient among the 19 patients successfully detected by visual assessment showed a comparable hyper-metabolism between the right hemisphere and the left hemisphere. However, nine patients showed worse abnormalities of glucose metabolism in the right hemisphere than the left hemisphere, while the other nine patients showed worse abnormalities of glucose metabolism in the left hemisphere than the right hemisphere.

On basal ganglia, 17 patients were identified as hyper-metabolism through visual assessment. However, the other 11 patients had been missed. Thus, the sensitivity of visual assessment on medial temporal lobe was about 61% (17/28). Five patients among the 17 patients successfully detected by visual assessment showed a comparable hyper-metabolism between the right hemisphere and the left hemisphere. However, four patients showed worse abnormalities of glucose metabolism in the right hemisphere than the left hemisphere, while the other seven patients showed worse abnormalities of glucose metabolism in the left hemisphere than the right hemisphere. Besides, one patient among the 17 patients showed the hyper-metabolism only in the left hemisphere.

Semi-quantitative Analysis Results

The results of semi-quantitative analysis were also shown in **Table 2**. On medial temporal lobe, 24 patients were identified as abnormal glucose metabolism. However, in the other four patients, the PET images didn't detect any abnormality. Thus, the sensitivity of semi-quantitative analysis on medial temporal lobe was about 86% (24/28).

On basal ganglia, 25 patients were identified as abnormal glucose metabolism. However, the other three patients weren't

TABLE 2 | Results of duration, autoantibody assay, visual assessment and semi-quantitative analysis.

Patient no.	Duration (month)	Autoantibody assay	Medial temporal lobe		Basal ganglia	
			Visual assessment	Semi-quantitative analysis	Visual assessment	Semi-quantitative analysis
1	6	Amphiphysin	NO	Left	NO	NO
2	1	$GABA_B$-R	NO	Bilateral	NO	Bilateral
3	1	$GABA_B$-R	Bilateral (R)	Bilateral	NO	Bilateral
4	1	$GABA_B$-R	Bilateral (R)	Bilateral	NO	Bilateral
5	8	$GABA_B$-R	NO	NO	NO	Bilateral*
6	2	LGI1	NO	Left* and Right	NO	Bilateral
7	0.5	LGI1	Bilateral (R)	Bilateral	Bilateral	Bilateral
8	0.5	LGI1	NO	Bilateral	Bilateral (R)	Bilateral
9	4	LGI1	Bilateral (L)	Bilateral	Bilateral (L)	Bilateral
10	3	LGI1	Bilateral (R)	Bilateral	Bilateral (L)	Bilateral
11	1	LGI1	Bilateral (R)	Bilateral	Bilateral	Bilateral
12	2	LGI1	Bilateral (R)	Bilateral	Bilateral (R)	Bilateral
13	10	LGI1	NO	NO	NO	NO
14	6	LGI1	Bilateral (L)	Left	NO	NO
15	6	LGI1	NO	NO	Bilateral	Bilateral
16	7	LGI1	NO	NO	NO	Right
17	3	LGI1	NO	Bilateral	NO	Left* and Right
18	3	LGI1	Bilateral (L)	Bilateral	NO	Left
19	3	LGI1	Bilateral	Bilateral	Bilateral (L)	Bilateral
20	1.5	LGI1	Bilateral (L)	Bilateral	Bilateral (R)	Bilateral
21	2	LGI1	Bilateral (R)	Bilateral	Bilateral (R)	Bilateral
22	2	LGI1	Bilateral (L)	Left	Left	Left and Right*
23	6	LGI1	Bilateral (R)	Bilateral	Bilateral (L)	Bilateral
24	2	LGI1	Bilateral (L)	Bilateral	Bilateral (L)	Bilateral
25	0.5	LGI1	Bilateral (L)	Bilateral	Bilateral	Bilateral
26	3	LGI1	Bilateral (L)	Bilateral	Bilateral (L)	Bilateral
27	1	LGI1	Bilateral (R)	Bilateral	Bilateral	Bilateral
28	1	LGI1	Bilateral (L)	Bilateral	Bilateral (L)	Bilateral

**The ROI was identified as hypometabolism.*

Bilateral (L/R) means that the level of hypermetabolism in left/right regions was higher than right/left regions.

identified as abnormality. Thus, the sensitivity of semi-quantitative analysis on basal ganglia was about 89% (25/28).

Comparison Between Semi-quantitative Analysis and Visual Assessment

On the medial temporal lobe, as shown in **Table 2**, 19 patients were identified as bilateral hyper-metabolism by visual assessment. All these patients were also successfully identified hyper-metabolism through semi-quantitative analysis, showing consistency with the visual assessment. The Kappa coefficient between visual and SPM analysis was 0.82. In addition, the lateralization of results from both analyses seemed no difference except two subjects. Subject 14 and 22 who were identified as bilateral hyper-metabolism on visual assessment were found only left medial temporal lobe hyper-metabolism by semi-quantitative analysis.

More importantly, five of nine patients who were not identified by visual inspection (56%) were detected by semi-quantitative analysis, demonstrating the greater sensitivity of semi-quantitative analysis compared with visual assessment. Three of these five patients were identified as a bilateral abnormality and one patient was found to be abnormal only on left medial temporal lobe, and the other patient was found hyper-metabolism on the right medial temporal lobe while hypo-metabolism on the left medial temporal lobe.

On the basal ganglia, as shown in **Table 2**, 17 patients were identified as bilateral hyper-metabolism by visual assessment. All of them were also identified hyper-metabolism through semi-quantitative analysis, showing consistency with the visual assessment. The Kappa coefficient between visual and SPM analysis was 0.71. The lateralization of results from both analysis seemed no difference. In addition, subject 22 who were identified as hyper-metabolism only on the left basal ganglia through visual assessment was found hyper-metabolism on the left basal ganglia while hypo-metabolism on the right basal ganglia through semi-quantitative analysis.

More importantly, eight of 11 patients not identified by visual inspection (73%) were detected by semi-quantitative analysis, demonstrating the greater sensitivity of semi-quantitative analysis compared with visual assessment. Four of these eight patients were identified as bilateral hyper-metabolism, and Four of these eight patients were identified as bilateral

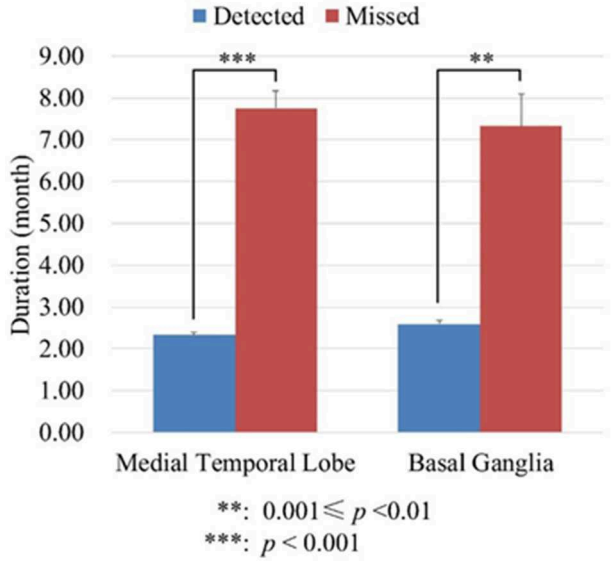

FIGURE 2 | The durations of disease (month) of detected and missed group patients. The detected group meant in these patients abnormal metabolism can be detected by semi-quantitative analysis. The missed group meant in these patients abnormal metabolism cannot be detected by semi-quantitative analysis.

hyper-metabolism, and one patient (subject 18) was found hyper-metabolism only on the left basal ganglia, and one patient (subject 16) was found hyper-metabolism only on the right basal ganglia, and one patient (subject 17) was found hyper-metabolism on the right basal ganglia while hypo-metabolism on the left basal ganglia, and the other one patient (subject 5) was found hypo-metabolism on the bilateral basal ganglia.

Although the sensitivity of semi-quantitative analysis increased significantly compared with visual inspection, it cannot detect abnormal metabolism in all those patients without abnormal metabolism by visual inspection. To know if disease duration was the interference factor, we divided the patients into two groups: in one group of patients abnormal metabolism can be detected (detected group) by semi-quantitative analysis; in the other group of patients abnormal metabolism cannot be detected (missed group) by semi-quantitative analysis. We analyzed the disease duration of the two groups of patients using the independent *t*-test. On the medial temporal lobe, the mean duration of detected group was 2.33 ± 1.71 months, whereas the mean duration of missed group was 7.75 ± 1.71 months. On the basal ganglia, the mean duration of detected group was 2.60 ± 2.10 months, whereas the mean duration of missed group was 7.33 ± 2.31 months. These results suggested that the patients without abnormal metabolism through semi-quantitative analysis had significantly longer disease duration (Seen in **Figure 2**).

DISCUSSION AND CONCLUSION

To date, FDG-PET/CT is one of largest increases in the numbers of medical imaging scanners (22). Moreover, FDG-PET/CT has been considered to be better than other conventional imaging tests in the clinical diagnostical settings, and it has showed good cost-effectiveness for non-small lung cancer staging (22). In addition, FDG-PET plays a key role in view of screening for occult malignancy for patients with paraneoplastic syndromes, including AE (23). Therefore, FDG-PET will probably become more commonly used inspection method for evaluating the suspicious AE patients in addition to malignancy screening (24). Many institutions apply a "vertex to toe" whole-body screening for malignancy. The additional 10 min dedicated brain 3D PET acquisition does not require extra radiopharmaceutical administration, which is easily brought into routine clinical workflows (24).

Since early immunotherapy can lead to better prognosis in AE, recent criteria have been improved to get early diagnosis (1). FDG-PET has been proposed to be a potentially useful diagnostic neuroimaging marker in suspected AE (8, 25). Some AE patients had normal mesial temporal lobe structures on MRI, whereas they showed hypermetabolism on FDG-PET (9, 26). These suggest that FDG-PET is more important than MRI in the early diagnosis and prognosis improvement of AE. Most previous studies of FDG-PET in AE only qualitatively described the FDG-PET findings (27–29). However, to date, there was lack of standard procedures for reading of FDG-PET imaging data in AE. Therefore, we sought to discuss the potential diagnostic benefit of semi-quantitative FDG-PET analysis compared with visual analysis by experienced neuroradiologists.

In the present study, we described semi-quantitative brain FDG-PET findings among patients with autoantibody positive AE, and compared this method with visual analysis at the same time. Our results showed that semi-quantitative brain FDG-PET analysis can find abnormal metabolism more sensitively, suggesting that semi-quantitative brain FDG-PET analysis was better than visual analysis. This study included a larger proportion of patients with LGI1. Previous case studies found striatal hyper-metabolism and/or the medial temporal lobe hyper-metabolism in patients with LGI1 encephalitis (1). However, the majority of these studies were described based on qualitative analyses only. Visual analysis is subjective and dependent on expertise, the level of experience can increase diagnostic accuracy of correlation with the clinical diagnosis, however, this effect was not affected using the analysis of SPM images (30, 31). Accurate visual analysis requires a good knowledge of normal distribution of F-18 FDG in various ages, characteristic distribution of metabolic abnormalities in various subtypes of AE, and normal brain anatomy and recognizing abnormal findings on low-dose CT scan and certain artifacts on PET/CT images. In the field of research in dementia, various semi-quantitative analysis programs have been developed over the years to detect mild abnormalities which are not apparent on visual inspection (32–34). Similar to dementia, it is urgent to develop various semi-quantitative analysis programs in AE. For the first time, we describe brain FDG-PET findings using semi-quantitative analysis among patients with autoantibody AE using a larger sample size.

The striatal hyper-metabolism in voltage-gated potassium channel-complex (VGKCc) encephalitis has been described previously (4, 35, 36) and may highly suggested the patients maybe positive for VGKCc antibodies, particularly in those

patients with faciobrachial dystonic seizures (4). Faciobrachial dystonic seizures seem to strongly linked to the leucine-rich glioma inactivated-1 (LGI1) protein target of VGKCc antibodies. Besides, hippocampus is the area where LGI1 protein most strongly expressed (37) superior to the striatum. Anti-$GABA_BR$ and anti-amphiphysin encephalitis as well as anti-LGI1 encephalitis all belong to limbic encephalitis. In addition, our previous study also showed that anti-$GABA_BR$ encephalitis and anti-amphiphysin encephalitis had similar clinical manifestation to anti-LGI1 encephalitis (3). Therefore, we chose medial temporal lobe and basal ganglia as ROI to observe the abnormalities of glucose metabolism. By our clinical observation, we found amygdala hyper-metabolism is more common in patients with LGI1. Besides, considering the size bias of the abnormalities in these areas, the present study not only examined the entire medial temporal lobe and basal ganglia, but also examined their fine-grained divisions. The fine-grained divisions can increase the sensitivity of finding abnormal metabolism. For example subject 2, we cannot detect abnormal glucose metabolism in entire medial temporal lobe, however, we can detect hyper-metabolism in right medial amygdala, bilateral lateral amygdala, and right rostral hippocampus.

Although semi-quantitative analysis can detect abnormal metabolism more sensitively compared with visual analysis, the positive rate can only reach 50% on medial temporal lobe and 67% on basal ganglia, respectively. We suppose that is due to the different disease duration. Previous studies showed that FDG-PET abnormal metabolism can change with disease evolution. Intense 18F-FDG uptake can be found in bilateral limbic system at active disease status, and then the 18F-FDG uptake decreased gradually and eventually returned to normal following the clinical improvement after treatment (12, 13). In addition, one study in regard to progressive primary aphasia patients who showed FDG-PET allowed researchers to detect abnormalities in the early stage of the disease (31). Our results also showed that the abnormal metabolism was associated with the disease status, which was consistent with the previous studies (12, 13, 31). This study was also the first quantitative descriptive FDG-PET study to certify that FDG-PET abnormal metabolism decreased following the prolongation of the disease duration. Therefore, the positive rate of semi-quantitative analysis may be higher than the present result if there are no significant disease duration difference among the patients.

Limitations of this study include the retrospective design, the significant different disease duration among the patients and relative small sample size of normal metabolism by visual analysis. Because abnormal metabolism was associated with different disease status, this will lead to misunderstandings of abnormal status. Although the whole sample size of AE was large, the patients with normal metabolism by visual analysis were relatively few, this need be evaluated by enrolling more patients in the future. Moreover, the patients were enrolled at a specialized tertiary epilepsy center, making the study subject to referral bias.

This study which showed semi-quantitative brain FDG-PET analysis was better than visual analysis in view of observing the abnormal glucose metabolism of patients with antibody positive AE. Semi-quantitative FDG-PET analysis appears to be a helpful tool in early diagnosis of patients with AE, especially those with normal MRI scans. Further research is needed to validate the sensitivity and specificity of semi-quantitative FDG-PET analysis in the early diagnosis of patients with AE. Besides, its usefulness for a better characterization of specific syndromes and their clinical course and response to therapy also needs to be further evaluated in prospective studies.

ETHICS STATEMENT

Informed consent to participate the study and for publication for clinical details were obtained from each subject enrolled. The study was approved by the Medical Ethics Committee of Beijing Tiantan Hospital, Capital Medical University and was carried out in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

R-JL and JL contributed to the study concept and design, and critically revised the manuscript for important intellectual content. R-JL, X-QS, QW, and X-BZ acquired the data. JP, GZ, JL, and R-JL analyzed and interpreted the data. R-JL, JP, and JL drafted the manuscript and provided statistical expertise. QW and X-QS provided administrative, technical, and material support. X-BZ and JL supervised the study.

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REFERENCES

1. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol*. (2016) 15:391–404. doi: 10.1016/S1474-4422(15)00401-9
2. Quek AM, Britton JW, McKeon A, So E, Lennon VA, Shin C, et al. Autoimmune epilepsy: clinical characteristics and response to immunotherapy. *Arch Neurol*. (2012) 69:582–93. doi: 10.1001/archneurol.2011.2985
3. Lv RJ, Ren HT, Guan HZ, Cui T, Shao XQ. Seizure semiology: an important clinical clue to the diagnosis of autoimmune epilepsy. *Ann Clin Transl Neurol*. (2018) 5:208–15. doi: 10.1002/acn3.520
4. Irani SR, Michell AW, Lang B, Pettingill P, Waters P, Johnson MR, et al. Faciobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. *Ann Neurol*. (2011) 69:892–900. doi: 10.1002/ana.22307
5. Titulaer MJ, McCracken L, Gabilondo I, Armangué T, Glaser C, Iizuka T, et al. Treatment and prognostic factors for long-termoutcome in patients with anti-

NMDA receptor encephalitis: an observational cohort study. *Lancet Neurol*. (2013) 12:157–65. doi: 10.1016/S1474-4422(12)70310-1
6. Arbizu J, Giuliani A, Gállego Perez-Larraya J, Riverol M, Jonsson C, García-García B, et al. Emerging clinical issues and multivariate analyses in PET investigations. *Q J Nucl Med Mol Imaging*. (2017) 61:386–404. doi: 10.23736/S1824-4785.17.03024-2
7. Gast H, Schindler K, Z'graggen WJ, Hess CW. Improvement of non-paraneoplastic voltage-gated potassium channel antibody-associated limbic encephalitis without immunosuppressive therapy. *Epilepsy Behav*. (2010) 17:555–7. doi: 10.1016/j.yebeh.2010.01.017
8. Morbelli S, Djekidel M, Hesse S, Pagani M, Barthel H, Neuroimaging Committee of the European Association of Nuclear Medicine (EANM), et al. Role of (18)F-FDG-PET imaging in the diagnosis of autoimmune encephalitis. *Lancet Neurol*. (2016) 15:1009–10. doi: 10.1016/S1474-4422(16)30140-5
9. Baumgartner A, Rauer S, Mader I, Meyer PT. Cerebral FDG-PET and MRI findings in autoimmune limbic encephalitis: correlation with autoantibody types. *J Neurol*. (2013) 260:2744–53. doi: 10.1007/s00415-013-7048-2
10. Newey CR, Sarwal A, Hantus S. [(18)F]-fluoro-deoxy-glucose positron emission tomography scan should be obtained early in cases of autoimmune encephalitis. *Autoimmune Dis*. (2016) 2016:9450452. doi: 10.1155/2016/9450452
11. Trevino-Peinado C, Arbizu J, Irimia P, Riverol M, Martínez-Vila E. Monitoring the effect of immunotherapy in autoimmune limbic encephalitis using 18F-FDG PET. *Clin Nucl Med*. (2015) 40:e441–3. doi: 10.1097/RLU.0000000000000839
12. Park S, Choi H, Cheon GJ, Wook Kang K, Lee DS. 18F-FDG PET/CT in anti-LGI1 encephalitis: initial and follow-up findings. *Clin Nucl Med*. (2015) 40:156–8. doi: 10.1097/RLU.0000000000000546
13. Kunze A, Drescher R, Kaiser K, Freesmeyer M, Witte OW, Axer H. Serial FDG PET/CT in autoimmune encephalitis with faciobrachial dystonic seizures. *Clin Nucl Med*. (2014) 39:e436–8. doi: 10.1097/RLU.0000000000000372
14. Yuan J, Guan H, Zhou X, Niu N, Li F, Cui L, et al. Changing brain metabolism patterns in patients with ANMDARE: serial 18F-FDG PET/CT findings. *Clin Nucl Med*. (2016) 41:366–70. doi: 10.1097/RLU.0000000000001164
15. Graus F, Dalmau J. Role of (18)F-FDG-PET imaging in the diagnosis of autoimmune encephalitis - Authors' reply. *Lancet Neurol*. (2016) 15:1010. doi: 10.1016/S1474-4422(16)30130-2
16. Morbelli S, Arbizu J, Booij J, Chen MK, Chetelat G, Cross DJ, et al. The need of standardization and of large clinical studies in an emerging indication of [(18)F]FDG PET: the autoimmune encephalitis. *Eur J Nucl Med Mol Imaging*. (2017) 44:353–357. doi: 10.1007/s00259-016-3589-9
17. Herholz K, Westwood S, Haense C, Dunn G. Evaluation of a calibrated (18)F-FDG PET score as a biomarker for progression in Alzheimer disease and mild cognitive impairment. *J Nucl Med*. (2011) 52:1218–26. doi: 10.2967/jnumed.111.090902
18. Yakushev I, Hammers A, Fellgiebel A, Schmidtmann I, Scheurich A, Buchholz HG, et al. SPM-based count normalization provides excellent discrimination of mild Alzheimer's disease and amnestic mild cognitive impairment from healthy aging. *Neuroimage*. (2009) 44:43–50. doi: 10.1016/j.neuroimage.2008.07.015
19. Moloney P, Boylan R, Elamin M, O'Riordan S, Killeen R, McGuigan C. Semi-quantitative analysis of cerebral FDG-PET reveals striatal hypermetabolism and normal cortical metabolism in a case of VGKCC limbic encephalitis. *Neuroradiol J*. (2017) 30:160–3. doi: 10.1177/1971400916689578
20. Pagani M, Öberg J, De Carli F, Calvo A, Moglia C, Canosa A, et al. Metabolic spatial connectivity in amyotrophic lateral sclerosis as revealed by independent component analysis. *Hum Brain Mapp*. (2016) 37:942–53. doi: 10.1002/hbm.23078
21. Fan L, Li H, Zhuo J, Zhang Y, Wang J, Chen L, et al. The human brainnetome atlas: a new brain atlas based on connectional architecture. *Cereb Cortex*. (2016) 26:3508–26. doi: 10.1093/cercor/bhw157
22. Buck AK, Herrmann K, Stargardt T, Dechow T, Krause BJ, Schreyögg J. Economic evaluation of PET and PET/CT in oncology: evidence and methodologic approaches. *J Nucl Med Technol*. (2010) 38:6–17. doi: 10.2967/jnmt.108.059584
23. McKeon A, Apiwattanakul M, Lachance DH, Lennon VA, Mandrekar JN, Boeve BF, et al. Positron emission tomography-computed tomography in paraneoplastic neurologic disorders: systematic analysis and review. *Arch Neurol*. (2010) 67:322–9. doi: 10.1001/archneurol.2009.336
24. Probasco JC, Solnes L, Nalluri A, Cohen J, Jones KM, Zan E, et al. Abnormal brain metabolism on FDG-PET/CT is a common early finding in autoimmune encephalitis. *Neurol Neuroimmunol Neuroinflamm*. (2017) 4:e352. doi: 10.1212/NXI.0000000000000352
25. Masangkay N, Basu S, Moghbel M, Kwee T, Alavi A. Brain 18F-FDG-PET characteristics in patients with paraneoplastic neurological syndrome and its correlation with clinical and MRI findings. *Nucl Med Commun*. (2014) 35:1038–46. doi: 10.1097/MNM.0000000000000163
26. Fisher RE, Patel NR, Lai EC, Schulz PE. Two different 18F-FDG brain PET metabolic patterns in autoimmune limbic encephalitis. *Clin Nucl Med*. (2012) 37:e213–8. doi: 10.1097/RLU.0b013e31824852c7
27. Lagarde S, Lepine A, Caietta E, Pelletier F, Boucraut J, Chabrol B, et al. Cerebral (18)FluoroDeoxy-Glucose positron emission tomography in paediatric anti N-methyl-D-aspartate receptor encephalitis: a case series. *Brain Dev*. (2016) 38:461–70. doi: 10.1016/j.braindev.2015.10.013
28. Kim TJ, Lee ST, Shin JW, Moon J, Lim JA, Byun JI, et al. Clinical manifestations and outcomes of the treatment of patients with GABAB encephalitis. *J Neuroimmunol*. (2014) 270:45–50. doi: 10.1016/j.jneuroim.2014.02.011
29. Shin YW, Lee ST, Shin JW, Moon J, Lim JA, Byun JI, et al. VGKC-complex/LGI1-antibody encephalitis: clinical manifestations and response to immunotherapy. *J Neuroimmunol*. (2013) 265:75–81. doi: 10.1016/j.jneuroim.2013.10.005
30. Morbelli S, Brugnolo A, Bossert I, Buschiazzo A, Frisoni GB, Galluzzi S, et al. Visual versus semi-quantitative analysis of 18F-FDG-PET in amnestic MCI: an European Alzheimer's Disease Consortium (EADC) project. *J Alzheimers Dis*. (2015) 44:815–26. doi: 10.3233/JAD-142229
31. Matias-Guiu JA, Cabrera-Martín MN, Pérez-Castejón MJ, Moreno-Ramos T, Rodríguez-Rey C, García-Ramos R, et al. Visual and statistical analysis of (1)(8)F-FDG PET in primary progressive aphasia. *Eur J Nucl Med Mol Imaging*. (2015) 42:916–27. doi: 10.1007/s00259-015-2994-9
32. Lehman VT, Carter RE, Claassen DO, Murphy RC, Lowe V, Petersen RC, et al. Visual assessment versus quantitative three-dimensional stereotactic surface projection fluorodeoxyglucose positron emission tomography for detection of mild cognitive impairment and Alzheimer disease. *Clin Nucl Med*. (2012) 37:721–6. doi: 10.1097/RLU.0b013e3182478d89
33. Gallivanone F, Della Rosa PA, Castiglioni I. Statistical voxel-based methods and [18F]FDG PET brain imaging: frontiers for the diagnosis of AD. *Curr Alzheimer Res*. (2016) 13:682–94. doi: 10.2174/1567205013666151116142039
34. Partovi S, Yuh R, Pirozzi S, Lu Z, Couturier S, Grosse U, et al. Diagnostic performance of an automated analysis software for the diagnosis of Alzheimer's dementia with (18)F FDG PET. *Am J Nucl Med Mol Imaging*. (2017) 7:12–23.
35. Kamaleshwaran KK, Iyer RS, Antony J, Radhakrishnan EK, Shinto A. 18F-FDG PET/CT findings in voltage-gated potassium channel limbic encephalitis. *Clin Nucl Med*. (2013) 38:392–4. doi: 10.1097/RLU.0b013e318286b58b
36. Wegner F, Wilke F, Raab P, Tayeb SB, Boeck AL, Haense C, et al. Anti-leucine rich glioma inactivated 1 protein and anti-N-methyl-D-aspartate receptor encephalitis show distinct patterns of brain glucose metabolism in 18F-fluoro-2-deoxy-d-glucose positron emission tomography. *BMC Neurol*. (2014) 14:136. doi: 10.1186/1471-2377-14-136
37. Irani SR, Alexander S, Waters P, Kleopa KA, Pettingill P, Zuliani L, et al. Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. *Brain*. (2010) 133:2734–48. doi: 10.1093/brain/awq213

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REVIEW
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Anti-Aβ Antibodies and Cerebral Amyloid Angiopathy Complications

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Cerebral amyloid angiopathy (CAA) corresponds to the deposition of amyloid material in the cerebral vasculature, leading to structural modifications of blood vessel walls. The most frequent form of sporadic CAA involves fibrillar β-amyloid peptide (Aβ) deposits, mainly the 40 amino acid form ($Aβ_{1-40}$), which are commonly found in the elderly with or without Alzheimer's disease. Sporadic CAA usually remains clinically silent. However, in some cases, acute complications either hemorrhagic or inflammatory can occur. Similar complications occurred after active or passive immunization against Aβ in experimental animal models exhibiting CAA, and in subjects with Alzheimer's disease during clinical trials. The triggering of these adverse events by active immunization and monoclonal antibody administration in CAA-bearing individuals suggests that analogous mechanisms could be involved during spontaneous CAA complications, drawing particular attention to the role of anti-Aβ antibodies. However, antibodies that react with several monomeric and aggregated forms of Aβ spontaneously occur in virtually all human individuals, hence being part of the "natural antibody" repertoire. Natural antibodies are usually described as having low-affinity and high cross-reactivity toward microbial components and autoantigens. Although frequently of the IgM class, they also belong to IgG and IgA isotypes. They likely display homeostatic functions and protective roles in aging. Until recently, the peculiar properties of these natural antibodies have hindered proper analysis of the Aβ-reactive antibody repertoire and the study of their implication in CAA complications. Herein, we review and comment the evidences of an auto-immune nature of spontaneous CAA complications, and discuss implications for forthcoming research and clinical practice.

Keywords: cerebral amyloid angiopathy, autoimmunity, anti-Aβ antibodies, immunotherapy, natural antibodies

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INTRODUCTION

Adaptive immunity in neurodegenerative conditions has recently become of major interest in neurodegenerative conditions, especially in Alzheimer's disease (AD), because of its ability to modulate cerebral inflammation, and also following observations that immunotherapeutic approaches might impair pathological events. However, the nature of immune effectors and their mechanisms remain quite obscure. In this article we will focus mainly on antibodies that bind the β-amyloid peptide (Aβ), which has long been considered a key therapeutic target. Evident difficulties are due to the presence of blood anti-Aβ antibodies in healthy and diseased individuals, implying that most quantitative studies provided little or no information on their pathological implication.

Another key point is that effector functions of these antibodies frequently are suggested to result in complications of cerebral amyloid angiopathy (CAA).

SPONTANEOUSLY OCCURRING ANTI-Aβ ANTIBODIES IN HEALTH AND DISEASE

Natural Antibodies: Origin, Distinctive Properties and Functions

Natural antibodies were defined as occurring in all normal individuals in the absence of overt antigenic stimulation (1). In spite of many controversies, it is now clear that natural antibodies represent a distinct entity that arises from separate B-cell lineages. Most natural antibodies are produced by a distinct B-cell lineage termed B1 cells, but also in part by marginal zone B cells that belong to the conventional B2 lineage. B1 cells originate very early from precursors of the yolk sac and then from hematopoietic stem cells of the fetal liver, but they also differentiate from bone marrow precursors during the adult life (2).

Classical adaptive B-cell responses to T-dependent antigens feature germinal center reactions that include affinity maturation and isotype switching, resulting in high affinity specific antibodies of IgG, IgA and/or IgE classes. These mechanisms allow improvement of effector efficiency as a function of antigen exposure. At variance with classical antibodies, natural antibodies likely arise from processes that involve no or little B-T cell cooperation and affinity maturation, as suggested by frequent absence or paucity of somatic hypermutations and nucleotide N-additions in the antibody variable regions. Thus, they display quite specific phenotypic properties as compared with conventional antibodies raised by immunization, including low binding affinities and multi-reactivity toward homologous antigens. Interestingly however, they may belong to IgG or IgA, as well as IgM classes (1, 3, 4).

In relation with these peculiar properties, it is thought that natural antibodies display specific functions. They likely protect from infection at early stages of development. In addition to supposed protective roles in the newborn, the ongoing production of natural antibodies has been proposed to interfere with pathological events of adults, such as tumors, atherosclerosis, and neurodegenerative disorders in elderly (5).

Natural IgM antibodies have long been studied in both mice and humans. They display a variety of functions, including pathogen neutralization and killing, and clearance of apoptotic cells and debris that participate in preventing inflammation in several pathological contexts. They also participate in maturation of adaptive responses, especially through antigen recruitment in secondary lymphoid tissues. Other well-known examples of IgM natural antibodies are isohemagglutinins.

Natural IgG and IgA antibodies are also abundant in the blood and mucosal secretions, in spite of the supposed absence of T cell help. Like IgM natural antibodies, they seem to play protective roles, especially in partnership with lectins such as Mannose-Binding Lectin and ficolins, against microbial pathogens and by controlling inflammation (3).

The repertoire of natural antibodies is clearly skewed, as they mostly recognize conserved self-structures (6, 7). Thus, they seem to undergo a selection process (8), suggesting physiological roles (9) including clearance of noxious molecules and cell debris, regulation of immune responses, and shaping of a primary repertoire that allows appropriate induced humoral responses. Numerous observations suggest that natural antibodies are protective against certain pathological conditions of aging, such as atherosclerosis, cancer and neurodegenerative disorders, and that the age-associated decreased expression of these antibodies could be responsible for proneness to these diseases (5). Indeed, circulating anti-alpha-synuclein antibody levels were found to be low in patients with Parkinson's disease (10). However, in AD, anti-Tau natural antibodies expression appears higher than in aged subjects (11). As discussed in the next section, the case of anti-Aβ antibodies and their expression in AD as compared to aged subjects remains controversial.

Natural Anti-Aβ Antibodies in Healthy Subjects, AD Patients and Related Conditions

Natural antibodies that react with Aβ peptide have long been suggested by the finding of specific B-cells (12), and their invariable presence was confirmed by studies comparing AD patients and healthy subjects (see below). As also found for other natural autoantibodies, aging results in progressive decrease of circulating anti-Aβ levels (13). They belong to both IgM and IgG classes, and predominant IgG subclasses appear to be IgG1 and IgG3 in both healthy and diseased subjects (14). They display a large panel of specificities for both linear and conformational epitopes of the Aβ molecules, oligomers, and fibrillar aggregates, and most interestingly, *in vitro* studies suggested that some natural anti-Aβ antibodies could protect neurons from toxic Aβ oligomers (15).

Analyses performed on serum or plasma from AD patients yielded remarkably incoherent results, as some of them revealed lower anti-Aβ antibody levels as compared with healthy controls (16–19), while others found higher levels in Alzheimer's disease patients (20–22) or no significant difference (13, 14, 23). Analyses performed on cerebrospinal fluid (CSF) led to similar discrepancies, displaying either lower (13, 16) or higher (20, 22) levels of anti-Aβ antibody in AD patients as compared with healthy controls. A common observation of those studies was the striking heterogeneity of antibody titers in healthy as well as in diseased individuals.

A first explanation for inconsistent results may be the diversity of methods used for antibody analysis: coated reactive antigens were $A\beta_{1-40}$ (16, 18, 19) or $A\beta_{1-42}$ (13, 17, 21, 23) in either oligomeric (13, 14, 18), fibrillar (20) or undefined isoforms (16, 17, 19, 21), at extremely variable concentrations; serum or plasma samples were tested either directly (17–20) or after dissociation of immune complexes (13, 14, 21); revealed antibodies were either IgG only (19) or all immunoglobulin isotypes (23); finally, a variety of different quantitation formulas were used. The only methodological agreement between recent studies is the requirement of an initial immune complex dissociation step for

a reliable analysis of anti-Aβ antibodies (13, 14, 21), using a mild acidic buffer in order to avoid artifacts (24). Indeed, a large part of circulating anti-Aβ antibodies appears to be associated with a variety of undefined ligands, likely due to polyreactivity and the presence of anti-idiotypic antibodies (15). A possible additional bias is that IgM antibodies have been demonstrated to potentially block natural IgG reactivity (25).

In addition to methodological matters, the other explanations of differences in anti-Aβ titers found between healthy and AD people relied on quite eclectic hypotheses: favored pathology due to decreasing antibody levels in aging (16), sequestering of antibodies by amyloid deposits (16), partial tolerization of B-cell responses (19) or decreased T-cell help (17), onset of an anti-Aβ autoimmune reaction (21, 22), or failure of immune complex clearance (22).

Natural Antibodies in Therapeutic Trials

That age-related decline of natural autoantibodies favors the onset of pathological conditions related to antigenic targets is supported by strong arguments (3). The hypothesis that natural anti-Aβ antibodies bear a strong protective potential (13, 26) has been the rationale for therapeutic trials in AD using human intravenous immunoglobulins (IVIg). Indeed, IVIg include natural antibodies to Aβ (27, 28) as well as to protein Tau (29). IVIg infusions appeared to increase the levels of circulating anti-Aβ (30), but while preliminary results on mild and moderate stages of AD had proven encouraging, more powerful trials failed to show a significant effect on cognitive decline (31–33). The reason of the lack of clinical efficacy of the natural anti-Aβ antibodies contained in IVIg is a matter of debate. It has been shown that IgG levels were elevated in the CSF of treated subjects. Thus, anti-Aβ antibodies penetrate into the CNS after infusion. Like other Aβ-modifying therapies, IVIg might be more effective in early stages of the disease. In addition, in these trials, IVIg were administered at low dose, similar to antibody replacement therapies. Immunomodulatory effects of IVIg are generally obtained in other neurological disease up to 2 g/kg (33).

ADAPTIVE IMMUNITY AGAINST Aβ: THE AN1792 TRIAL

Histological response and cognitive improvement after Aβ vaccination in transgenic mouse models of AD have been received enthusiastically in the late 1990's (34, 35). Elan/Wyeth-Ayerst Pharmaceuticals set up the AN1792 trial in human (NCT00021723), the phase I study of safety and immunogenicity began in 1999, Dec., vaccinating mild-to-moderate AD subjects with pre-aggregated $A\beta_{1-42}$ with adjuvant QS-21, and later on with QS-21 plus polysorbate-80 0.4%. After no major safety issue, phase IIa (Study 201) began in 2001, Oct., and was halted in 2002, Jan., when some patients presented with meningoencephalitis (36). Other adverse events other than meningoencephalitis occurred in the AN1792 study: two cases of encephalopathy including one without meningoencephalitis, two cases of convulsion, one thrombosis of the retina, one hemiplegia, and, interestingly, one case of intracerebral hemorrhage (ICH) related to CAA. However, confronted with the striking manifestations of brain inflammation, these post-immunization hemorrhagic or ischemic events have been overlooked (37).

Overall, 18 out of 300 (6%) subjects treated with AN1792 in phase II developed meningoencephalitis, along with 1 subject from the extended phase I study (37–40). Clinically, these patients presented with subacute aseptic meningoencephalitis with confusion, lethargy, and headaches. Other symptoms were nausea, seizures, drowsiness, ataxia, aphasia, hemiparesia, with inconstant fever. As in aseptic meningoencephalitis, CSF analysis showed mild to moderate hyperlymphocytosis (3-130 cells/mm^3), often with mild hyperproteinorachia (39). Since none of the placebo-treated group developed such serious adverse events, it was obvious that these cases of meningoencephalitis related to the active immunization against Aβ.

Only 2 patients with post-Aβ immunization meningoencephalitis were investigated at the histological level. The first patient (40) presented with fever, drowsiness, and unstable gait. At neuroimaging, MRI found white matter hyperintensities (WMH) and a more focal lesion, possibly associated with edema and inflammatory process. Post-mortem tissue sampling showed cortical areas without senile plaques, but still with CAA, and lymphocytic infiltration of leptomeningeal Aβ-laden blood vessels, or in perivascular space around cortical vessels, with sparse cellular infiltration of the parenchyma. Cellular infiltrates were mainly composed of CD4+ CD45RO+ T-cells, with few CD8+ T-cells, and without CD79a+ CD20+ B-cells. The second patient (41) presented similar plaque-devoid cortical areas and CD8+ T-cell-enriched perivascular lymphocytic infiltrates. In addition, the authors describe multiple macroscopic cortical hemorrhages that were not detected radiologically despite 3 MRI studies, and microscopic vasculopathy, namely lipohyalinosis. Interestingly, a third case (42) reported pathological findings in an AN1792-treated patient without clinical meningoencephalitis, which also presented perivascular cellular infiltrates in the leptomeningeal spaces, though to a lower extent as compared with the meningoencephalitis cases, and composed mainly of CD20+ B-cells and few CD3+ T-cells.

The immune mechanism, underlying these cases of meningoencephalitis has been debated, though it seemed obvious that adaptive immunity against Aβ was at stake.

In the AN1792 trial, treated-patients were considered either antibody responder or non-responder with respect to the circulating anti-Aβ antibody titer supposed to confer clinical benefit (38). While 20% treated patients were considered antibody responders, most patients (at least 90%) seemed to have produced detectable levels of circulating IgM and/or IgG anti-Aβ after immunization (37). Among treated-patients with meningoencephalitis, 13 out of 18 belonged to the responder group, and 5 out of 18 to the non-responder group. Among these 5 non-responders, all of them had a measurable anti-Aβ IgM response, and 4 a measurable anti-Aβ IgG response in the blood (38).

If almost all treated patients developed an anti-Aβ antibody response, one could question whether the antibody response was qualitatively different in patients with meningoencephalitis.

However, it was shown by epitope-mapping that all immunized patients recognized a linear B-cell epitope in the N-terminus of the Aβ peptide ($A\beta_{1-8}$), independently of the occurrence of meningoencephalitis (43), and that their serum antibodies bound to parenchymal as well as vascular Aβ aggregates, and not to cellular APP (44).

Out of 57 studied cases, 9 (15%) had measurable CSF IgG or IgM anti-Aβ antibody levels and 4 presented with meningoencephalitis (3 responders and 1 non-responder) (38). However, CSF antibodies from both patients with or without meningoencephalitis bound to senile plaques and vascular Aβ aggregates (44).

T-cell responses during the AN1792 trial have been poorly studied. One paper reports on the higher occurrence of IFNγ producing cells after $A\beta_{1-42}$ stimulation in phase IIa treated-patients as compared to phase I treated-patients, which was not significantly different from the placebo-treated group (45).

Confronted to the absence of noticeable differences in the anti-Aβ antibody responses in AN1792-treated patients with and without meningoencephalitis, and considering the presence of lymphocytic infiltrates during such cases, it was assumed that the adaptive anti-Aβ cellular T-cell response was detrimental when oriented toward cellular immunity through Th1 CD4+ and cytotoxic CD8+ T-cells, while anti-Aβ B-cell response would remain innocuous and potentially beneficial for AD pathology. Thus, subsequent anti-Aβ immunotherapy trials were based on anti-Aβ antibodies either by selective "humoral" vaccines or, most frequently, by passive immunization using repeated humanized monoclonal antibody infusions, the complications of which have been linked to CAA.

CEREBRAL AMYLOID ANGIOPATHY AND RELATED CLINICAL MANIFESTATIONS

Definition, Epidemiology, and Neuropathological Features of Cerebral Amyloid Angiopathy

Deposited amyloid material in the blood vessel walls of the cerebral vasculature is made up of insoluble fibrils resulting from the polymerization of peptidic subunits with a "crossed β-sheet"-rich conformation. Mutations of several proteins have been described in familial forms of CAA, such as Aβ precursor protein (APP) and other proteins (46). In addition to mutations, duplications of the wild-type *APP* gene are also associated with CAA, as found in Down syndrome and in autosomal dominant early onset Alzheimer's disease (47). Sporadic CAA, which is an Aβ-related disease without any mutation or duplication of the *APP* gene, represents the most common form of CAA and will be the main focus of this review.

The main risk factors of sporadic CAA are advancing age and the co-occurrence of AD (48, 49). Both frequency and severity of CAA increase with age, affecting 35, 50, and 75% of individuals in the 7th, 8th and 9th decades of life, respectively, regardless of CAA severity (49). Population-based studies show that in age-matched groups, demented individuals have higher risk to present CAA than non-demented individuals, regardless of CAA severity or considering solely severe CAA (50). Other genetic risk factors have been described, some of them overlapping with sporadic AD risk factors, making it difficult to distinguish which ones are independently related to sporadic CAA. The main genetic risk factor is ApoE polymorphism: the ε4 allele is associated with the occurrence of AD and CAA, while the ε2 allele appears protective against AD but associated with the risk of CAA-related intra-cerebral hemorrhage (ICH) (51). To explain these controversial findings, Greenberg et al. hypothesized that the APOE2 allele might be related to CAA vasculopathy but not vascular deposits, which they evidenced by showing that the APOE2 allele is more frequent in patients with grade 3-4 CAA (cracking of the vessel wall and paravascular leaking of blood) but that it is not over-represented in grade 2 CAA (complete amyloid replacement of vessels) (52). Hence, APOE4 might promote a higher risk of CAA-related hemorrhage by enhancing amyloid deposition, while APOE2 might cause amyloid-laden vessels to undergo vasculopathic changes that lead to their rupture.

It is indeed important to stress that, from a pathological point of view, CAA does not stand for the mere presence of fibrillary Aβ deposition in blood vessel walls. More severe vascular deposits are associated with vasculopathies, such as fibrinoid necrosis, thrombosis, microhemorrhages, or "double-barreling," which is the concentric splitting of the vessel walls (53). Associated with smooth muscles cell degeneration and amyloid replacement of the whole vessel wall structure, these pathological features provide the histologic ground for clinical and paraclinical features of the manifestations of CAA. Cerebrovascular amyloid deposition in sporadic CAA involves the leptomeningeal and cortical medium- and small-sized arteries/arterioles, with or without involvement of the capillaries, and usually sparing the veins. The topography of CAA is not homogenous through the brain. The most affected areas being the occipital, parietal, frontal, then temporal lobes. In most advanced cases, other structures than the neocortex can be affected. It is now thought that some CAA features, such as capillary involvement, dyshoric angiopathy hence small vessel disease occurring by capillary occlusion, are associated with AD pathology, while leptomeningeal and arteriolar involvement is mainly independent from parenchymal amyloidosis of AD pathology (49). This could lead to a reappraisal of ApoE polymorphism in CAA: the ε4 allele would be associated with AD capillary CAA, while the ε2 allele would be associated with arterial CAA and its specific manifestations, such as lobar ICH (51).

CAA Manifestations

Cerebrovascular impairment in sporadic CAA may result in 3 subsets of manifestations: ischemia, bleeding, and inflammation (54).

Ischemic manifestations of CAA are cortical infarcts and WMH appearing on the MRI. They predominate in posterior regions of the brain where CAA accumulates, and relate to hypoperfusion or vessel occlusion (55–57).

Hemorrhagic manifestations of CAA are symptomatic ICH that differentiates from hypertensive ICH by strictly lobar localization, corresponding to the leptomeningeal and cortical

topography of CAA (58). Besides symptomatic ICH, cortical microbleeds (59) and superficial cortical siderosis are other hemorrhagic features of CAA that can be displayed on MRI (60). Both ischemic and hemorrhagic features of CAA contribute to chronic vascular dementia.

Inflammatory manifestations of CAA involve a protean clinical presentation of corticosensitive encephalopathy, e.g., with subacute cognitive decline, seizures, headaches, and asymmetric T2 WMH. Histopathologicaly, different inflammatory subtypes have been described associated with CAA: (i) Aβ-related angiitis, which corresponds to destructive transmural infiltrates with granulomatous features; (ii) CAA-related inflammation (CAA-ri), which corresponds to non-destructive perivascular lymphocytic infiltrates. However, besides histopathological phenotypes, clinical features, imaging and outcome largely overlap, making these entities likely part of the same spectrum (61, 62). These pathologies represent up to 30% of primary CNS vasculitis, making it part of single-organ vasculitis in the Chappell-Hill revised nomenclature (63, 64), and introducing the hypothesis of an auto-immune mechanism of these manifestations.

With the noticeable exception of extremely rare CAA-ri cases, the potential auto-immune mechanisms at stake in CAA manifestations have been neglected. Clinical and imaging features of CAA-related hemorrhages or ischemia have been conceived as the consequence of mechanical constrains, blood wall weakening, mural degeneration and or vessel occlusion related to vascular Aβ deposition.

However, it has been convincingly shown that CAA was increased in patients immunized with AN1792. In a post-mortem study of 9 treated-patients, Boche et al. found a worsening of CAA in AN1792 patients, with cerebrovascular deposits containing not only $A\beta_{1-40}$, which is classically found in CAA, but also higher cerebrovascular levels of $A\beta_{1-42}$, the main component of senile plaques. These patients also had a higher rate of vasculopathies and microhemorrhages (65). In our opinion, and as suggested by some authors (66, 67), the possibility cannot be discarded that detrimental adaptive immunity raised against Aβ during the AN1792, whether humoral or cellular, was as least partly related to cerebrovascular deposits, given: (i) the predominant leptomeningeal and perivascular location of cellular infiltrates in meningoencephalitis cases during the AN1792 trial, (ii) the occurrence of some post-immunization hemorrhagic or ischemic severe adverse events, including one case of CAA-related ICH, and (iii) the multiple cortical hemorrhages and associated lipohyalinosis described by Ferrer et al. in one meningoencephalitis case (41).

Whether meningoencephalitis and other adverse events were related to adaptive immune reaction against parenchymal or against vascular Aβ deposits, it was assumed that T-cell anti-Aβ response was detrimental, while humoral adaptive anti-Aβ response was thought to be inocuous and potentially beneficial. However, adverse events also occurred in the course of passive immunotherapies with anti-Aβ antibodies, which shed a new light on the pathophysiology of induced and spontaneously-occurring CAA manifestations.

MONOCLONAL ANTI-Aβ ANTIBODIES AND THEIR RELATIONSHIP TO CAA MANIFESTATIONS

The Illustrative Case of Bapineuzumab

Bapineuzumab is a monoclonal humanized IgG1 antibody corresponding to the murine clone 3D6, targeting the N-terminus of the Aβ peptide ($A\beta_{1-5}$). However, unlike circulating antibodies arising in AN1792 patients, Bapineuzumab does not recognize a linear epitope but a conformational one (68). While patients treated by Bapineuzumab showed a decrease of the cerebral amyloid load by 11C-PiB TEP-scan (69, 70), they did not show clinical improvement (71).

During the phase I trial, 3 out of 10 patients treated with a single infusion of the highest dose of Bapineuzumab (5 mg/kg) developed transient signal abnormalities on T2*/fluid attenuation inversion recovery (FLAIR) sequences, interpreted as a sign of vasogenic cerebral edema (72). Additional cases were reported during phase II (73) and phase III (74). First referred as vasogenic edema, these amyloid-related imaging abnormalities (ARIA) were included in a spectrum of so-called ARIA-E (E standing for effusion), that encompasses increased MR signal intensity on FLAIR or other T2-weighted sequences in the parenchyma gray or white matter and/or leptomeninges, sometimes in the cerebellum or brainstem. Interestingly, this radiological presentation appears similar to MRI presentation of spontaneous inflammatory manifestations of CAA (66, 74). ARIA-E was found in 17% (36/210) Bapineuzumab-treated subjects, appearing after the first or second infusion, in a dose-dependent manner, with an increased risk in ApoE ε4 carriers (7% among non-carriers, 18% among heterozygous, 36% among homozygous) (71). ARIA-E was associated with clinical manifestations during Bapineuzumab trials in about 20% subjects. If acute clinical worsening prompted off-protocol MRI in some cases, most patients were detected by per-protocol MRI. However, some were retrospectively found to present clinical signs, similar to inflammatory manifestations of CAA. Another type of ARIA, namely ARIA-H (H standing for hemorrhages) appeared during the Bapineuzumab studies in 12% patients, and featured either microbleeds or cortical superficial siderosis similar to spontaneous hemorrhagic manifestations of CAA. ARIA-E and ARIA-H are not independent phenomena. First, the presence of baseline microbleeds increased the risk of ARIA-E. Second, *de novo* ARIA-H were 10 times more prevalent in patients with ARIA-E (67). They appeared during the same timeline, and often in the same cortical areas (66).

Although no neuropathological description of ARIA-E have been reported, the pathophysiological relationship between ARIA-E and spontaneous CAA-related inflammation on the one hand, and ARIA-H, and spontaneous CAA-related hemorrhages on the other hand, was suggested. Hence, it became obvious that antibodies targeting Aβ are sufficient to trigger CAA-like manifestations, and that T-cell anti-Aβ responses arising from active immunization are not required. Thus, some authors suggested that despite differences in severity, meningoencephalitis and ARIA-E could be part of

a same pathophysiological spectrum linked to Aβ vascular deposition (66, 67).

ARIA in Other Anti-Aβ Passive Immunotherapies

Aducanumab is a human IgG1 selected from a B-cell library obtained from healthy aged donors who were cognitively normal. It was selected for its reactivity against soluble Aβ oligomers and insoluble Aβ fibrils. Similarly to Bapineuzumab, 22% (27/125) Aducanumab-treated subjects developed ARIA-E, 33% being symptomatic, usually mild. Once again, this phenomenon appeared to be dose-dependent and ApoE ε4-related. Among ARIA-E subjects, 15 (56%) also had ARIA-H. In particular, while none of the placebo-treated subjects had cortical superficial siderosis, 9 Aducanumab-treated patients presented with such manifestations (75).

Gantenerumab is a human IgG1 selected by phage display from a human antibody library and optimized *in vitro* to enhance its affinity to fibrillar Aβ, and binds to the N-terminal $Aβ_{1-10}$ but also the middle portion $Aβ_{19-26}$ (76). Then again, 10% (53/531) treated patients developed ARIA-E, and 20% (104/531) developed ARIA-H in a dose-dependent, ApoE ε4-related manner. The clinical course was similar to those of Bapineuzumab and Aducanumab in terms of incidence, symptoms, kinetics and response to withdrawal (77).

Solanezumab is a humanized IgG1 that binds to the mid-domain of soluble Aβ. ARIA-E occurred in 18 Solanezumab-treated subjects, however it was always asymptomatic, with a low incidence (1.1%), and without statistical difference from placebo-treated subjects (0.5%). Under Solanezumab treatment, ARIA-H was more frequent than ARIA-E (9.1%), but once again without statistically significant difference from placebo-treated subjects (7.3%). Even though Solanezumab did not seem to induce a higher frequency of ARIA, the link between ARIA-E and ARIA-H was still obvious: 71% of patients with ARIA-E displayed increases in ARIA-H at the time of ARIA-E, and 48% had co-localized ARIA-H and ARIA-E (78).

Crenezumab is a humanized IgG4 that was designed in order to avoid FcγRc-binding, hence anti-Aβ antibody-mediated cellular reaction in order to lower the risk of ARIA. Crenezumab has a preferential affinity for soluble oligomers (79). Crenezumab-treated patients in the ABBY study did not show a higher incidence of ARIA-E, microbleeds and cortical superficial siderosis. Of note, one asymptomatic macrohemorrhage was reported in a treated-patient (80). In the independent BLAZE study, however, 14.5% treated-patients presented new ARIA-H, as compared to 3.4% placebo-treated patients (81).

Ponezumab is a humanized IgG2 targeting the C-terminus of $Aβ_{30-40}$ that binds to soluble Aβ. The incidence of microhemorrhages was 16.4% in the Ponezumab group and 21.4% in the placebo group. However, various MRI features corresponding to what can be observed during CAA were reported solely in the Ponezumab-treated group, either inflammatory-like (1 case with ARIA-E associated with superficial siderosis, 1 case with cerebral/meningeal enhancement), hemorrhagic-like (1 case of subdural hematoma), or ischemic-like (4 cases of cortical infarcts, 2 subcortical gray-matter infarcts) (82). In 24 CAA patients treated with Ponezumab, 2 hemorrhagic adverse events (1 case of cerebral hemorrhage, 1 case of subdural hematoma), and 1 inflammatory-like event (1 case of asymptomatic ARIA-E) occurred but were not considered treatment-related by the investigators (83).

Other antibodies have been used in Aβ-related diseases. AAB-003 was developed by introducing a 3-aminoacid mutation in the Fc region of Bapineuzumab, resulting in reduced FcγRc binding and reduced binding to C1q. ARIA-H and ARIA-E were however reported in the treated group (84). BAN2401 is a humanized IgG1 monoclonal antibody that selectively binds to Aβ soluble protofibrils and that did not appear to be related with ARIA during phase I (85).

CONCLUSION: IMPLICATIONS FOR THE MANAGEMENT OF CAA COMPLICATIONS

Overall, treatments of Aβ-related diseases with monoclonal anti-Aβ antibodies can trigger CAA-like manifestations, either inflammatory or hemorrhagic. In most cases, these radiological signs are not associated with clinical manifestations. Since they were followed by treatment discontinuation in most trials, the question of whether the asymptomatic radiological signs would have remained infra-clinical or represent a pre-clinical state remains unanswered. The mechanism of such CAA-like manifestations is also unknown. Anti-Aβ antibodies could simply worsen CAA (65) by displacing parenchymal Aβ to the cerebral vasculature, hence enhancing the risk of CAA manifestations. A more direct pathogenic role for anti-Aβ antibodies would involve the mediation of inflammation and/or the triggering of hemorrhagic events after antibody binding to cerebrovascular deposits, by impairment of the structural integrity of the blood vessel, complement-mediated or FcγRc-mediated inflammation. As a general rule, three concomitant factors seem to modulate the risk of ARIA. The dose-dependent effect of such anti-Aβ monoclonal antibodies is the first and better established one. The second one is anti-Aβ antibody isotype: IgG1 anti-Aβ monoclonal antibodies such as Bapineuzumab, Aducanumab, and Gantenerumab, triggered ARIA-E and ARIA-H, while IgG2 (Ponezumab) and IgG4 (Crenezumab) appeared less prone to trigger CAA-like manifestations. The third one is the selectivity for soluble or deposited forms of Aβ: Ponezumab and Crenezumab were more selective for targeting soluble forms of Aβ, as were Solanezumab and BAN2401, hence maybe less prone than other antibodies to bind to cerebrovascular deposits in body fluids.

In any case, the pathogenicity of anti-Aβ antibodies likely depends on their characteristics: isotype (Ig class and subclass), specificity toward soluble or aggregated forms of $Aβ_{1-42}$ or $Aβ_{1-40}$, differential affinity for such forms, and concentration in body fluids.

By analogy with passive immunotherapy-induced ARIA, these considerations lead to the hypothesis that in sporadic CAA, the occurrence of some species of anti-Aβ antibodies can trigger spontaneous inflammatory or hemorrhagic CAA manifestations. However, serum anti-Aβ antibodies have been scarcely studied in

CAA (86). Most investigations focused on (i) CSF antibodies (ii) during CAA-related inflammation. Piazza et al. showed elevated CSF levels of anti-Aβ IgG during the clinical and radiological active phase of spontaneous CAA-ri (87). The same group argued in favor of an intrathecal synthesis of CSF anti-Aβ antibodies, based on a single case (88). However, experimental evidence in transgenic mouse model that exhibit AD-like pathology with a strong CAA component suggested that blood anti-Aβ antibodies are by themselves prone to aggravate CAA and related manifestations, including hemorrhages (89, 90).

Anti-Aβ antibodies circulating in the serum are part of the natural antibody repertoire. Despite methodological difficulties hindering their study, adverse events that occurred during anti-Aβ immunotherapies in AD suggest that natural anti-Aβ antibodies are likely to play a role in the triggering of CAA-related inflammation, but also in other CAA manifestations such as hemorrhagic features. Whether such pathogenic anti-Aβ species would be part of an individual pre-immune natural repertoire, or would be the result of adaptive processes driven by cerebrovascular deposits during the course of CAA is unknown. The pathogenic features of these anti-Aβ antibodies that would correlate with CAA clinical phenotypes remain to be determined by a fine-scale analysis of the circulating anti-Aβ antibody repertoire, focusing on serum concentration, isotypes and IgG subclasses, specificity, and affinity toward soluble or aggregated forms of Aβ. Such analysis would deliver pathophysiological insights about CAA manifestations, but would hopefully provide new biomarkers for the prediction of spontaneous and immunotherapy-induced complications in sporadic CAA and AD.

To date, cognition and education remain the best predictors of dementia in the elderly, while ApoE polymorphisms consistently predict Aβ-related pathology (91). The vascular component in AD and other dementia has raised more and more interest over the last years. In this view, the study of spontaneously produced pathogenic anti-Aβ antibodies seems promising. Its inclusion in prediction models of dementia might be the keystone that would link cerebrovascular Aβ deposition and macro- or microvascular impairment.

AUTHOR CONTRIBUTIONS

YC and PA wrote the manuscript. All authors revised the manuscript.

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REFERENCES

1. Coutinho A, Kazatchkine MD, Avrameas S. Natural autoantibodies. *Curr Opin Immunol*. (1995) 7:812-8. doi: 10.1016/0952-7915(95)80053-0
2. Montecino-Rodriguez E, Dorshkind K. B-1 B cell development in the fetus and adult. *Immunity*. (2012) 36:13-21. doi: 10.1016/j.immuni.2011.11.017
3. Panda S, Ding JL. Natural antibodies bridge innate and adaptive immunity. *J Immunol*. (2015) 194:13-20. doi: 10.4049/jimmunol.1400844
4. Baccala R, Quang TV, Gilbert M, Ternynck T, Avrameas S. Two murine natural polyreactive autoantibodies are encoded by nonmutated germ-line genes. *Proc Natl Acad Sci USA*. (1989) 86:4624-8. doi: 10.1073/pnas.86.12.4624
5. Rothstein TL. Natural antibodies as rheostats for susceptibility to chronic diseases in the aged. *Front Immunol*. (2016) 7:127. doi: 10.3389/fimmu.2016.00127
6. Mouthon L, Nobrega A, Nicolas N, Kaveri SV, Barreau C, Coutinho A, et al. Invariance and restriction toward a limited set of self-antigens characterize neonatal IgM antibody repertoires and prevail in autoreactive repertoires of healthy adults. *Proc Natl Acad Sci USA*. (1995) 92:3839-43. doi: 10.1073/pnas.92.9.3839
7. Mouthon L, Haury M, Lacroix-Desmazes S, Barreau C, Coutinho A, Kazatchkine MD. Analysis of the normal human IgG antibody repertoire. Evidence that IgG autoantibodies of healthy adults recognize a limited and conserved set of protein antigens in homologous tissues. *J Immunol*. (1995) 154:5769-78.
8. Hardy RR, Hayakawa K. Selection of natural autoreactive B cells. *Clin Exp Rheumatol*. (2015) 33(4 suppl. 92):S80-6.
9. Nagele EP, Han M, Acharya NK, DeMarshall C, Kosciuk MC, Nagele RG. Natural IgG autoantibodies are abundant and ubiquitous in human sera, and their number is influenced by age, gender, and disease. *PLoS ONE*. (2013) 8:e60726. doi: 10.1371/journal.pone.0060726
10. Besong-Agbo D, Wolf E, Jessen F, Oechsner M, Hametner E, Poewe W, et al. Naturally occurring α-synuclein autoantibody levels are lower in patients with Parkinson disease. *Neurology*. (2013) 80:169-75. doi: 10.1212/WNL.0b013e31827b90d1
11. Rosenmann H, Meiner Z, Geylis V, Abramsky O, Steinitz M. Detection of circulating antibodies against tau protein in its unphosphorylated and in its neurofibrillary tangles-related phosphorylated state in Alzheimer's disease and healthy subjects. *Neurosci Lett*. (2006) 410:90-3. doi: 10.1016/j.neulet.2006.01.072
12. Gaskin F, Finley J, Fang Q, Xu S, Fu SM. Human antibodies reactive with beta-amyloid protein in Alzheimer's disease. *J Exp Med*. (1993) 177:1181-6. doi: 10.1084/jem.177.4.1181
13. Britschgi M, Olin CE, Johns HT, Takeda-Uchimura Y, LeMieux MC, Rufibach K, et al. Neuroprotective natural antibodies to assemblies of amyloidogenic peptides decrease with normal aging and advancing Alzheimer's disease. *Proc Natl Acad Sci USA*. (2009) 106:12145-50. doi: 10.1073/pnas.0904866106
14. Dorothée G, Bottlaender M, Moukari E, de Souza LC, Maroy R, Corlier F, et al. Distinct patterns of antiamyloid-β antibodies in typical and atypical Alzheimer disease. *Arch Neurol*. (2012) 69:1181-5. doi: 10.1001/archneurol.2012.604
15. Szabo P, Relkin N, Weksler ME. Natural human antibodies to amyloid beta peptide. *Autoimmun Rev*. (2008) 7:415-20. doi: 10.1016/j.autrev.2008.03.007
16. Du Y, Dodel R, Hampel H, Buerger K, Lin S, Eastwood B, et al. Reduced levels of amyloid beta-peptide antibody in Alzheimer disease. *Neurology*. (2001) 57:801-5. doi: 10.1212/WNL.57.5.801
17. Weksler ME, Relkin N, Turkenich R, LaRusse S, Zhou L, Szabo P. Patients with Alzheimer disease have lower levels of serum anti-amyloid peptide antibodies than healthy elderly individuals. *Exp Gerontol*. (2002) 37:943-8. doi: 10.1016/S0531-5565(02)00029-3
18. Moir RD, Tseitlin KA, Soscia S, Hyman BT, Irizarry MC, Tanzi RE. Autoantibodies to redox-modified oligomeric Abeta are attenuated in the plasma of Alzheimer's disease patients. *J Biol Chem*. (2005) 280:17458-63. doi: 10.1074/jbc.M414176200
19. Jianping L, Zhibing Y, Wei Q, Zhikai C, Jie X, Jinbiao L. Low avidity and level of serum anti-Abeta antibodies in Alzheimer disease. *Alzheimer Dis Assoc Disord*. (2006) 20:127-32. doi: 10.1097/00002093-200607000-00001
20. Nath A, Hall E, Tuzova M, Dobbs M, Jons M, Anderson C, et al. Autoantibodies to amyloid ß-peptide (Aß) are increased in Alzheimer's disease patients and Aß antibodies can enhance Aß neurotoxicity: implications for disease pathogenesis and vaccine development. *Neuromolecular Med*. (2003) 3:29-39 doi: 10.1385/NMM:3:1:29

21. Storace D, Cammarata S, Borghi R, Sanguineti R, Giliberto L, Piccini A, et al. Elevation of {beta} amyloid 1-42 autoantibodies in the blood of amnestic patients with mild cognitive impairment. *Arch Neurol*. (2010) 67:867-72. doi: 10.1001/archneurol.2010.137
22. Maftei M, Thurm F, Schnack C, Tumani H, Otto M, Elbert T, et al. Increased levels of antigen-bound β-amyloid autoantibodies in serum and cerebrospinal fluid of Alzheimer's disease patients. *PLoS ONE*. (2013) 8:e68996. doi: 10.1371/journal.pone.0068996
23. Hyman BT, Smith C, Buldyrev I, Whelan C, Brown H, Tang MX, et al. Autoantibodies to amyloid-beta and Alzheimer's disease. *Ann Neurol*. (2001) 49:808-10. doi: 10.1002/ana.1061
24. Li Q, Gordon M, Cao C, Ugen KE, Morgan D. Improvement of a low pH antigen-antibody dissociation procedure for ELISA measurement of circulating anti-Abeta antibodies. *BMC Neurosci*. (2007) 8:22. doi: 10.1186/1471-2202-8-22
25. Adib M, Ragimbeau J, Avrameas S, Ternynck T. IgG autoantibody activity in normal mouse serum is controlled by IgM. *J Immunol*. (1990) 145:3807-13.
26. Kellner A, Matschke J, Bernreuther C, Moch H, Ferrer I, Glatzel M. Autoantibodies against beta-amyloid are common in Alzheimer's disease and help control plaque burden. *Ann Neurol*. (2009) 65:24-31. doi: 10.1002/ana.21475
27. Dodel R, Hampel H, Depboylu C, Lin S, Gao F, Schock S, et al. Human antibodies against amyloid beta peptide: a potential treatment for Alzheimer's disease. *Ann Neurol*. (2002) 52:253-6. doi: 10.1002/ana.10253
28. O'Nuallain B, Hrncic R, Wall JS, Weiss DT, Solomon A. Diagnostic and therapeutic potential of amyloid-reactive IgG antibodies contained in human sera. *J Immunol*. (2006) 176:7071-8. doi: 10.4049/jimmunol.176.11.7071
29. Hromadkova L, Kolarova M, Jankovicova B, Bartos A, Ricny J, Bilkova Z, et al. Identification and characterization of natural antibodies against tau protein in an intravenous immunoglobulin product. *J Neuroimmunol*. (2015) 289:121-9. doi: 10.1016/j.jneuroim.2015.10.017
30. Relkin NR, Szabo P, Adamiak B, Burgut T, Monthe C, Lent RW, et al. 18-Month study of intravenous immunoglobulin for treatment of mild Alzheimer disease. *Neurobiol Aging*. (2009) 30:1728-36. doi: 10.1016/j.neurobiolaging.2007.12.021
31. Dodel R, Rominger A, Bartenstein P, Barkhof F, Blennow K, Förster S, et al. Intravenous immunoglobulin for treatment of mild-to-moderate Alzheimer's disease: a phase 2, randomised, double-blind, placebo-controlled, dose-finding trial. *Lancet Neurol*. (2013) 12:233-43. doi: 10.1016/S1474-4422(13)70014-0
32. Kile S, Au W, Parise C, Rose K, Donnel T, Hankins A, et al. IVIG treatment of mild cognitive impairment due to Alzheimer's disease: a randomised double-blinded exploratory study of the effect on brain atrophy, cognition and conversion to dementia. *J Neurol Neurosurg Psychiatry*. (2017) 88:106-112. doi: 10.1136/jnnp-2015-311486
33. Relkin NR, Thomas RG, Rissman RA, Brewer JB, Rafii MS, van Dyck CH, et al. A phase 3 trial of IV immunoglobulin for Alzheimer disease. *Neurology*. (2017) 88:1768-75. doi: 10.1212/WNL.0000000000003904
34. Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature*. (1999) 400:173-7. doi: 10.1038/22124
35. Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, et al. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature*. (2000) 408:982-5. doi: 10.1038/35050116
36. Check E. Nerve inflammation halts trial for Alzheimer's drug. *Nature*. (2002) 415:462. doi: 10.1038/415462a
37. Bayer AJ, Bullock R, Jones RW, Wilkinson D, Paterson KR, Jenkins L, et al. Evaluation of the safety and immunogenicity of synthetic Abeta42 (AN1792) in patients with AD. *Neurology*. (2005) 64:94-101. doi: 10.1212/01.WNL.0000148604.77591.67
38. Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC, et al. Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. *Neurology*. (2005) 64:1553-62. doi: 10.1212/01.WNL.0000159740.16984.3C
39. Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, et al. Subacute meningoencephalitis in a subset of patients with AD after Abeta42 immunization. *Neurology*. (2003) 61:46-54. doi: 10.1212/01.WNL.0000073623.84147.A8
40. Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat Med*. (2003) 9:448-52. doi: 10.1038/nm840
41. Ferrer I, Boada Rovira M, Sánchez Guerra ML, Rey MJ, Costa-Jussá F. Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. *Brain Pathol*. (2004) 14:11-20. doi: 10.1111/j.1750-3639.2004.tb00493.x
42. Masliah E, Hansen L, Adame A, Crews L, Bard F, Lee C, et al. Abeta vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease. *Neurology*. (2005) 64:129-31. doi: 10.1212/01.WNL.0000148590.39911.DF
43. Lee M, Bard F, Johnson-Wood K, Lee C, Hu K, Griffith SG, et al. Abeta42 immunization in Alzheimer's disease generates Abeta N-terminal antibodies. *Ann Neurol*. (2005) 58:430-5. doi: 10.1002/ana.20592
44. Hock C, Konietzko U, Papassotiropoulos A, Wollmer A, Streffer J, von Rotz RC, et al. Generation of antibodies specific for beta-amyloid by vaccination of patients with Alzheimer disease. *Nat Med*. (2002) 8:1270-5. doi: 10.1038/nm783
45. Pride M, Seubert P, Grundman M, Hagen M, Eldridge J, Black RS. Progress in the active immunotherapeutic approach to Alzheimer's disease: clinical investigations into AN1792-associated meningoencephalitis. *Neurodegener Dis*. (2008) 5:194-6. doi: 10.1159/000113700
46. Revesz T, Holton JL, Lashley T, Plant G, Frangione B, Rostagno A, et al. Genetics and molecular pathogenesis of sporadic and hereditary cerebral amyloid angiopathies. *Acta Neuropathol*. (2009) 118:115-30. doi: 10.1007/s00401-009-0501-8
47. Cabrejo L, Guyant-Maréchal L, Laquerrière A, Vercelletto M, De la Fournière F, Thomas-Antérion C, et al. Phenotype associated with APP duplication in five families. *Brain*. (2006) 129:2966-76. doi: 10.1093/brain/awl237
48. Vinters HV, Gilbert JJ. Cerebral amyloid angiopathy: incidence and complications in the aging brain. II. The distribution of amyloid vascular changes. *Stroke*. (1983) 14:924-8. doi: 10.1161/01.STR.14.6.924
49. Thal DR, Griffin WS, de Vos RA, Ghebremedhin E. Cerebral amyloid angiopathy and its relationship to Alzheimer's disease. *Acta Neuropathol*. (2008) 115:599-609. doi: 10.1007/s00401-008-0366-2
50. Keage HA, Carare RO, Friedland RP, Ince PG, Love S, Nicoll JA, et al. Population studies of sporadic cerebral amyloid angiopathy and dementia: a systematic review. *BMC Neurol*. (2009) 9:3. doi: 10.1186/1471-2377-9-3
51. Yamada M. Cerebral amyloid angiopathy: emerging concepts. *J Stroke*. (2015) 17:17-30. doi: 10.5853/jos.2015.17.1.17
52. Greenberg SM, Vonsattel JP, Segal AZ, Chiu RI, Clatworthy AE, Liao A, et al. Association of apolipoprotein E epsilon2 and vasculopathy in cerebral amyloid angiopathy. *Neurology*. (1998) 50:961-5. doi: 10.1212/WNL.50.4.961
53. Love S, Chalmers K, Ince P, Esiri M, Attems J, Jellinger K, et al. Development, appraisal, validation and implementation of a consensus protocol for the assessment of cerebral amyloid angiopathy in post-mortem brain tissue. *Am J Neurodegener Dis*. (2014) 3:19-32.
54. Charidimou A, Boulouis G, Gurol ME, Ayata C, Bacskai BJ, Frosch MP, et al. Emerging concepts in sporadic cerebral amyloid angiopathy. *Brain*. (2017) 140:1829-50. doi: 10.1093/brain/awx047
55. Zhu YC, Chabriat H, Godin O, Dufouil C, Rosand J, Greenberg SM, et al. Distribution of white matter hyperintensity in cerebral hemorrhage and healthy aging. *J Neurol*. (2012) 259:530-6. doi: 10.1007/s00415-011-6218-3
56. Thanprasertsuk S, Martinez-Ramirez S, Pontes-Neto OM, Ni J, Ayres A, Reed A, et al. Posterior white matter disease distribution as a predictor of amyloid angiopathy. *Neurology*. (2014) 83:794-800. doi: 10.1212/WNL.0000000000000732
57. Gurol ME, Viswanathan A, Gidicsin C, Hedden T, Martinez-Ramirez S, Dumas A, et al. Cerebral amyloid angiopathy burden associated with leukoaraiosis: a positron emission tomography/magnetic resonance imaging study. *Ann Neurol*. (2013) 73:529-36. doi: 10.1002/ana.23830
58. Hirohata M, Yoshita M, Ishida C, Ikeda SI, Tamaoka A, Kuzuhara S, et al. Clinical features of non-hypertensive lobar intracerebral hemorrhage related to cerebral amyloid angiopathy. *Eur J Neurol*. (2010) 17:823-9. doi: 10.1111/j.1468-1331.2009.02940.x
59. Cordonnier C, Al-Shahi Salman R, Wardlaw J. Spontaneous brain microbleeds: systematic review, subgroup analyses and standards for

study design and reporting. *Brain*. (2007) 130:1988-2003. doi: 10.1093/brain/awl387
60. Linn J, Halpin A, Demaerel P, Ruhland J, Giese AD, Dichgans M, et al. Prevalence of superficial siderosis in patients with cerebral amyloid angiopathy. *Neurology*. (2010) 74:1346-50. doi: 10.1212/WNL.0b013e3181dad605
61. Salvarani C, Hunder GG, Morris JM, Brown RD Jr, Christianson T, Giannini C. Aβ-related angiitis: comparison with CAA without inflammation and primary CNS vasculitis. *Neurology*. (2013) 81:1596-603. doi: 10.1212/WNL.0b013e3182a9f545
62. Salvarani C, Brown RD Jr, Christianson T, Miller DV, Giannini C, Huston J III, et al. An update of the Mayo Clinic cohort of patients with adult primary central nervous system vasculitis: description of 163 patients. *Medicine*. (2015) 94:e738. doi: 10.1097/MD.0000000000000738
63. Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, et al. 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. *Arthritis Rheum*. (2013) 65:1-11. doi: 10.1002/art.37715
64. Salvarani C, Brown RD Jr, Hunder GG. Adult primary central nervous system vasculitis. *Lancet*. (2012) 380:767-77. doi: 10.1016/S0140-6736(12)60069-5
65. Boche D, Zotova E, Weller RO, Love S, Neal JW, Pickering RM, et al. Consequence of Abeta immunization on the vasculature of human Alzheimer's disease brain. *Brain*. (2008) 131:3299-310. doi: 10.1093/brain/awn261
66. Sperling RA, Jack CR Jr, Black SE, Frosch MP, Greenberg SM, Hyman BT, et al. Amyloid-related imaging abnormalities in amyloid-modifying therapeutic trials: recommendations from the Alzheimer's Association Research Roundtable Workgroup. *Alzheimers Dement*. (2011) 7:367-85. doi: 10.1016/j.jalz.2011.05.2351
67. Frisoni GB. ARIA from off-key operas? *Lancet Neurol*. (2012) 11:207-8. doi: 10.1016/S1474-4422(12)70021-2
68. Miles LA, Crespi GA, Doughty L, Parker MW. Bapineuzumab captures the N-terminus of the Alzheimer's disease amyloid-beta peptide in a helical conformation. *Sci Rep*. (2013) 3:1302. doi: 10.1038/srep01302
69. Rinne JO, Brooks DJ, Rossor MN, Fox NC, Bullock R, Klunk WE, et al. 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. *Lancet Neurol*. (2010) 9:363-72. doi: 10.1016/S1474-4422(10)70043-0
70. Liu E, Schmidt ME, Margolin R, Sperling R, Koeppe R, Mason NS, et al. Amyloid-β 11C-PiB-PET imaging results from 2 randomized bapineuzumab phase 3 AD trials. *Neurology*. (2015) 85:692-700. doi: 10.1212/WNL.0000000000001877
71. Salloway S, Sperling R, Fox NC, Blennow K, Klunk W, Raskind M, et al. Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *N Engl J Med*. (2014) 370:322-33. doi: 10.1056/NEJMoa1304839
72. Black RS, Sperling RA, Safirstein B, Motter RN, Pallay A, Nichols A, et al. A single ascending dose study of bapineuzumab in patients with Alzheimer disease. *Alzheimer Dis Assoc Disord*. (2010) 24:198-203. doi: 10.1097/WAD.0b013e3181c53b00
73. Salloway S, Sperling R, Gilman S, Fox NC, Blennow K, Raskind M, et al. A phase 2 multiple ascending dose trial of bapineuzumab in mild to moderate Alzheimer disease. *Neurology*. (2009) 73:2061-70. doi: 10.1212/WNL.0b013e3181c67808
74. Sperling R, Salloway S, Brooks DJ, Tampieri D, Barakos J, Fox NC, et al. Amyloid-related imaging abnormalities in patients with Alzheimer's disease treated with bapineuzumab: a retrospective analysis. *Lancet Neurol*. (2012) 11:241-9. doi: 10.1016/S1474-4422(12)70015-7
75. Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, et al. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. *Nature*. (2016) 537:50-6. doi: 10.1038/nature19323
76. Panza F, Solfrizzi V, Imbimbo BP, Giannini M, Santamato A, Seripa D, et al. Efficacy and safety studies of gantenerumab in patients with Alzheimer's disease. *Expert Rev Neurother*. (2014) 14:973-86. doi: 10.1586/14737175.2014.945522
77. Ostrowitzki S, Lasser RA, Dorflinger E, Scheltens P, Barkhof F, Nikolcheva T, et al. A phase III randomized trial of gantenerumab in prodromal Alzheimer's disease. *Alzheimers Res Ther*. (2017) 9:95. doi: 10.1186/s13195-017-0318-y
78. Carlson C, Siemers E, Hake A, Case M, Hayduk R, Suhy J, et al. Amyloid-related imaging abnormalities from trials of solanezumab for Alzheimer's disease. *Alzheimers Dement*. (2016) 2:75-85. doi: 10.1016/j.dadm.2016.02.004
79. Adolfsson O, Pihlgren M, Toni N, Varisco Y, Buccarello AL, Antoniello K, et al. An effector-reduced anti-β-amyloid (Aβ) antibody with unique aβ binding properties promotes neuroprotection and glial engulfment of Aβ. *J Neurosci*. (2012) 32:9677-89. doi: 10.1523/JNEUROSCI.4742-11.2012
80. Cummings JL, Cohen S, van Dyck CH, Brody M, Curtis C, Cho W, et al. ABBY: A phase 2 randomized trial of crenezumab in mild to moderate Alzheimer disease. *Neurology*. (2018) 90:e1889-97. doi: 10.1212/WNL.0000000000005550
81. Salloway S, Honigberg LA, Cho W, Ward M, Friesenhahn M, Brunstein F, et al. Amyloid positron emission tomography and cerebrospinal fluid results from a crenezumab anti-amyloid-beta antibody double-blind, placebo-controlled, randomized phase II study in mild-to-moderate Alzheimer's disease (BLAZE). *Alzheimers Res Ther*. (2018) 10:96. doi: 10.1186/s13195-018-0424-5
82. Landen JW, Cohen S, Billing CB Jr, Cronenberger C, Styren S, Burstein AH, et al. Multiple-dose ponezumab for mild-to-moderate Alzheimer's disease: Safety and efficacy. *Alzheimers Dement*. (2017) 3:339-47. doi: 10.1016/j.trci.2017.04.003
83. Leurent C, Goodman JA, Zhang Y, He P, Polimeni JR, Gurol ME, et al. Immunotherapy with ponezumab for probable cerebral amyloid angiopathy. *Ann Clin Transl Neurol*. (2019) 6:795-806. doi: 10.1002/acn3.761
84. Delnomdedieu M, Duvvuri S, Li DJ, Atassi N, Lu M, Brashear HR, et al. First-In-Human safety and long-term exposure data for AAB-003 (PF-05236812) and biomarkers after intravenous infusions of escalating doses in patients with mild to moderate Alzheimer's disease. *Alzheimers Res Ther*. (2016) 8:12. doi: 10.1186/s13195-016-0177-y
85. Logovinsky V, Satlin A, Lai R, Swanson C, Kaplow J, Osswald G et al. Safety and tolerability of BAN2401-a clinical study in Alzheimer's disease with a protofibril selective Aβ antibody. *Alzheimers Res Ther*. (2016) 8:14. doi: 10.1186/s13195-016-0181-2
86. Cao Z, Lv J, Quan W. Low avidity and level of serum anti-Aβ antibodies in patients with cerebral amyloid angiopathy-related cerebral hemorrhage. *Int J Neurosci*. (2010) 120:760-4. doi: 10.3109/00207454.2010.520379
87. Piazza F, Greenberg SM, Savoiardo M, Gardinetti M, Chiapparini L, Raicher I, et al. Anti-amyloid β autoantibodies in cerebral amyloid angiopathy-related inflammation: implications for amyloid-modifying therapies. *Ann Neurol*. (2013) 73:449-58. doi: 10.1002/ana.23857
88. DiFrancesco JC, Brioschi M, Brighina L, Ruffmann C, Saracchi E, Costantino G, et al. Anti-Aβ autoantibodies in the CSF of a patient with CAA-related inflammation: a case report. *Neurology*. (2011) 76:842-4. doi: 10.1212/WNL.0b013e31820e773c
89. Pfeifer M, Boncristiano S, Bondolfi L, Stalder A, Deller T, Staufenbiel M, et al. Cerebral hemorrhage after passive anti-Abeta immunotherapy. *Science*. (2002) 298:1379. doi: 10.1126/science.1078259
90. Thakker DR, Weatherspoon MR, Harrison J, Keene TE, Lane DS, Kaemmerer WF, et al. Intracerebroventricular amyloid-beta antibodies reduce cerebral amyloid angiopathy and associated micro-hemorrhages in aged Tg2576 mice. *Proc Natl Acad Sci USA*. (2009) 106:4501-6. doi: 10.1073/pnas.0813404106
91. Hall A, Pekkala T, Polvikoski T, van Gils M, Kivipelto M, Lötjönen J, et al. Prediction models for dementia and neuropathology in the oldest old: the Vantaa 85+ cohort study. *Alzheimers Res Ther*. (2019) 11:11. doi: 10.1186/s13195-018-0450-3

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Common Neurodegeneration-Associated Proteins Are Physiologically Expressed by Human B Lymphocytes and Are Interconnected via the Inflammation/Autophagy-Related Proteins TRAF6 and SQSTM1

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There is circumstantial evidence that, under neurodegenerative conditions, peptides deriving from aggregated or misfolded specific proteins elicit adaptive immune responses. On another hand, several genes involved in familial forms of neurodegenerative diseases exert key innate immune functions. However, whether or not such observations are causally linked remains unknown. To start addressing this issue, we followed a systems biology strategy based on the mining of large proteomics and immunopeptidomics databases. First, we retrieved the expression patterns of common neurodegeneration-associated proteins in two professional antigen-presenting cells, namely B lymphocytes and dendritic cells. Surprisingly, we found that under physiological conditions, numerous neurodegeneration-associated proteins are abundantly expressed by human B lymphocytes. A survey of the human proteome allowed us to map a unique protein-protein interaction network linking common neurodegeneration-associated proteins and their first shell interactors in human B lymphocytes. Interestingly, network connectivity analysis identified two major hubs that both relate with inflammation and autophagy, namely TRAF6 (TNF Receptor Associated Factor 6) and SQSTM1 (Sequestosome-1). Moreover, the mapped network in B lymphocytes comprised two additional hub proteins involved in both inflammation and autoimmunity: HSPA8 (Heat Shock Protein Family A Member 8 also known as HSC70) and HSP90AA1 (Heat Shock Protein 90 Alpha Family Class A Member 1). Based on these results, we then explored the Immune Epitope Database "IEDB-AR" and actually found that a large share of neurodegeneration-associated proteins were previously reported to provide endogenous MHC class II-binding peptides in human B lymphocytes. Of note, peptides deriving from amyloid beta A4 protein, sequestosome-1 or profilin-1 were reported to bind multiple allele-specific MHC class II molecules. In

contrast, peptides deriving from microtubule-associated protein tau, presenilin 2 and serine/threonine-protein kinase TBK1 were exclusively reported to bind MHC molecules encoded by the HLA-DRB1 1501 allele, a recently-identified susceptibility gene for late onset Alzheimer's disease. Finally, we observed that the whole list of proteins reported to provide endogenous MHC class II-binding peptides in human B lymphocytes is specifically enriched in neurodegeneration-associated proteins. Overall, our work indicates that immunization against neurodegeneration-associated proteins might be a physiological process which is shaped, at least in part, by B lymphocytes.

Keywords: neurodegeneration, bioinformatics, autoimmunity, B-lymphocytes, amyloid-beta-protein, tau & phospho-tau protein, synuclein, prion

INTRODUCTION

Multiple studies have now established that neurodegenerative disorders are not cell-autonomous. The pathophysiological processes leading to neurodegeneration involve and target not only neurons but also glial cells, including astrocytes, microglia, and oligodendrocytes (1–3). Moreover, beyond central nervous system (CNS) cells, the adaptive immune system has emerged as a potentially important player. When considering only T-cell responses, T-cell reactivity against amyloid beta peptides was already reported more than a decade ago in both patients suffering from Alzheimer's disease (AD) and aged healthy subjects (4). Recent works further demonstrated that alpha-synuclein (SNCA)-derived peptides elicit helper and cytotoxic T-cell responses in a subgroup of Parkinson's disease (PD) patients harboring specific major histocompatibility complex (MHC) alleles (5, 6). Similarly, in animal models of neurodegenerative disorders, T-cell responses against peptides deriving from neurodegeneration-associated proteins were also demonstrated. In particular, in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model of PD, pathogenic brain-infiltrating T-cells directed against SNCA were found to target the substantia nigra of diseased mice (7, 8). Along this line, in a mouse model of tauopathy, CD8 cytotoxic T-cells directed against tau protein were shown to infiltrate the hippocampus and to drive cognitive alterations (9). Finally, besides AD and PD, several studies provided evidence that T-cells are activated during the course of Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) and/or their animal models (10–13). Nevertheless, the putative autoantigens targeted under those conditions remain unknown.

Extending the functional results mentioned above, genome-wide association studies (GWAS) have unraveled the central position of autoimmunity-related genes in the genetic susceptibility landscape of neurodegenerative diseases (14–19). This is notably the case for *HLA-DRB1* alleles which were recently demonstrated to confer increased risks of developing PD (15) or late-onset AD (14). Also, two genes involved in familial forms of PD, namely *PINK1* (PTEN-induced putative kinase 1) and *PRKN* (Parkin RBR E3 Ubiquitin Protein Ligase also named PARK2 or Parkin) were shown to regulate the presentation of mitochondria-derived antigens in the context of MHC class I molecules (20). Finally, innate immune functions were demonstrated for several genes causatively-linked to familial forms of PD or ALS. These comprise *C9ORF72* (21–23), *LRRK2* (Leucine-rich repeat kinase 2) (24–27), *GRN* (Granulin Precursor) (28, 29) as well as *PRKN* and *PINK1* (30–34).

However, advocating for the role of autoimmunity in neurodegenerative disorders requires yet addressing several important issues. In particular, it appears crucial to determine how T-cells directed against neurodegeneration-associated antigens are primed in the periphery. The extent to which autoimmunity against neurodegeneration-associated antigens might be a physiological event needs also to be assessed. Last but not least, a global view on the expression of neurodegeneration-associated proteins by professional antigen-presenting cells (APCs), in particular B lymphocytes and dendritic cells, is still lacking. In an attempt to address these issues, we used here a systems biology approach embracing a large range of previously-published experimental data. We notably explored in normal human B lymphocytes and dendritic cells the expression patterns of the most common neurodegeneration-associated proteins. Our data mining results indicate that in human B lymphocytes, a large majority of neurodegeneration-associated proteins are expressed at the protein level. Moreover, a survey of the human proteome unravels that neurodegeneration-associated proteins expressed by B lymphocytes may form a complex network centered on the inflammation/autophagy-related molecules SQSTM1 (Sequestosome-1) and TRAF6 (TNF Receptor Associated Factor 6). Finally, the analysis of MHC class II immunopeptidome databases provides evidence that neurodegeneration-associated proteins expressed by human B lymphocytes are a source of endogenous peptides which are presented in the context of HLA class II molecules.

MATERIALS AND METHODS

Workflow

A scheme summarizing the workflow followed in the present work is shown in **Figure 1**.

Data Mining Methods and Bioinformatics Tools

All the bioinformatics analyses were performed at least 3 times between March 2018 and October 2019. Databases, bioinformatic tools, and corresponding tasks performed in this study are described below.

FIGURE 1 | Workflow of the study. The workflow starts from the upper central gray rectangle. Other rectangles (in red) frame the main results obtained following each of the analytical steps which are briefly described in green ellipse shapes. Terms in italics correspond to the name of the bioinformatics tools used for each analytical step. MHC: major histocompatibility complex.

- The Genetics Home Reference website (35), is a regularly updated consumer health resource from the National Library of Medicine. It provides information to the general public about the effects of genetic variation on human health. In the present paper, we used the "Genetics Home Reference" website to select without *a priori* the most common genetic variations/alterations that are linked to the following neurodegenerative disorders: Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, fronto-temporal dementia (FTD).
- The enrichment web platform Enrichr (36) performs enrichment analyses from queried lists of genes. The Enrichr website allows surveying simultaneously 132 libraries gathering 245,575 terms and their associated lists of genes or proteins. Enrichment analysis tools provided by the Enrichr bioinformatics platform provides adjusted *P*-values computed from the Fisher's exact test. We focused our analysis on the "Jensen DISEASES" ontology library (37) which is based exclusively on text-mining and allow determining whether a list of genes is significantly associated with specific disease-related terms.
- The BioGrid database (38) compiles 29 169 experimentally-proven protein-protein interactions connecting 23 098 human proteins. Querying the BioGrid database allows retrieving for any given human protein the current list of published experimentally-identified human protein partners.
- The Human Proteome Map database (39) compiles protein expression data obtained by mass spectrometry from human normal tissues and cells including a total of 85 human blood samples from which the protein profiles of 6 blood-circulating cell types were established. In parallel, we also explored 3 recently-published mass spectrometry datasets reporting on the protemics profiles of blood-derived human B lymphocytes (40), blood-derived human dendritic cells (DCs) (41) and cultured monocyte-derived human dendritic cells (MoDCs) (42).
- The Immune Epitope Database and Analysis Resource (IEDB-AR) (43) compiles experimental data on antibody and T-cell epitopes in humans, non-human primates and other animal species. We followed a 3 step strategy as described below.

  Step 1: retrieval of a list of human MHC class II binding peptides and their parent proteins
  - in the "Epitope" tab, the "Any Epitopes" item was marked
  - in the "Assay" tab, the "MHC Ligand Assays" item was marked
  - in the "Antigen" tab, "Homo sapiens" was entered in the "Organism" line
  - in the "MHC restriction" tab, the "MHC class II" item was marked
  - in the "Host" tab, the "Humans" item was marked
  - in the "Disease" tab, the "Any Disease" item was marked.

  Search was then launched and, from the results page, the "Assays" tab was selected and a list of currently known ligands (peptides and parent proteins) of human MHC molecules was retrieved.

  Step 2: filtering the results

  We retained only results which were both: (i) obtained by mass spectrometry analysis of peptides eluted from MHC class II molecules and (ii) generated from cells of the B- cell lineage, in the absence of immunization or stimulation protocols.

  Step 3: checking the reference IDs associated to each identified parent protein

  For each selected study and/or set of results, the reference ID associated to each parent protein was checked on the UniProt website (44) in order to avoid redundant or obsolete IDs.
- The Cytoscape software (45) is an open source software allowing to visualize complex networks. We used the function "Network analysis" to identify hubs i.e., objects exhibiting the highest number of degrees (connections to other objects) in the generated networks.

Statistics

When not embedded in bioinformatics webtools, statistics for enrichment analyses were performed using the Fisher's exact test. In particular, we assessed for several identified hub proteins whether their retrieved list of partners (obtained via the BioGrid database) was statistically enriched in neurodegeneration-associated proteins. To calculate enrichment factors, we set the expected reference ratio as 0.002 which corresponds to the number of common neurodegeneration-associated proteins (i.e., 48 according to the "Home Genetics Reference" website) over the number of coding genes for which interactors can be retrieved from the BioGrid database (i.e., 23098). The obtained *p*-values were then adjusted using Bonferroni correction. The same approach was used to determine whether lists of parent proteins from which derive human MHC class II-binding peptides are significantly enriched in neurodegeneration-associated proteins.

RESULTS

Neurodegeneration-Associated Proteins Form a Unique Interaction Network in Which PRKN Is the Main Hub

From the most recently updated version of the human proteome compiled in the "BioGrid" database, we extracted the currently published and experimentally-proven protein-protein interactions connecting the most common neurodegeneration-associated proteins. Self-interactions were excluded from our analysis. From these retrieved interactions we were able to build and visualize a protein network which encompasses 35 (72%) of the 48 most common neurodegeneration-associated proteins (**Figure 2**). Interestingly, network analysis showed that PRKN harbored the highest number of interacting partners ($n = 18$). Moreover, when considering the whole list of known PRKN protein partners, the calculated enrichment factor in neurodegeneration associated protein reached 20.86 and was highly significant (Fisher's exact test *p*-value: $2.77E^{-16}$). It should be noticed that the great majority of the protein-protein interactions reported so far between PRKN and other common neurodegeneration-associated proteins were demonstrated via robust low throughput biochemical approaches as detailed in **Data Supplement 1**. Our data mining result points thus to a yet unnoticed property of PRKN as a major hub protein connecting

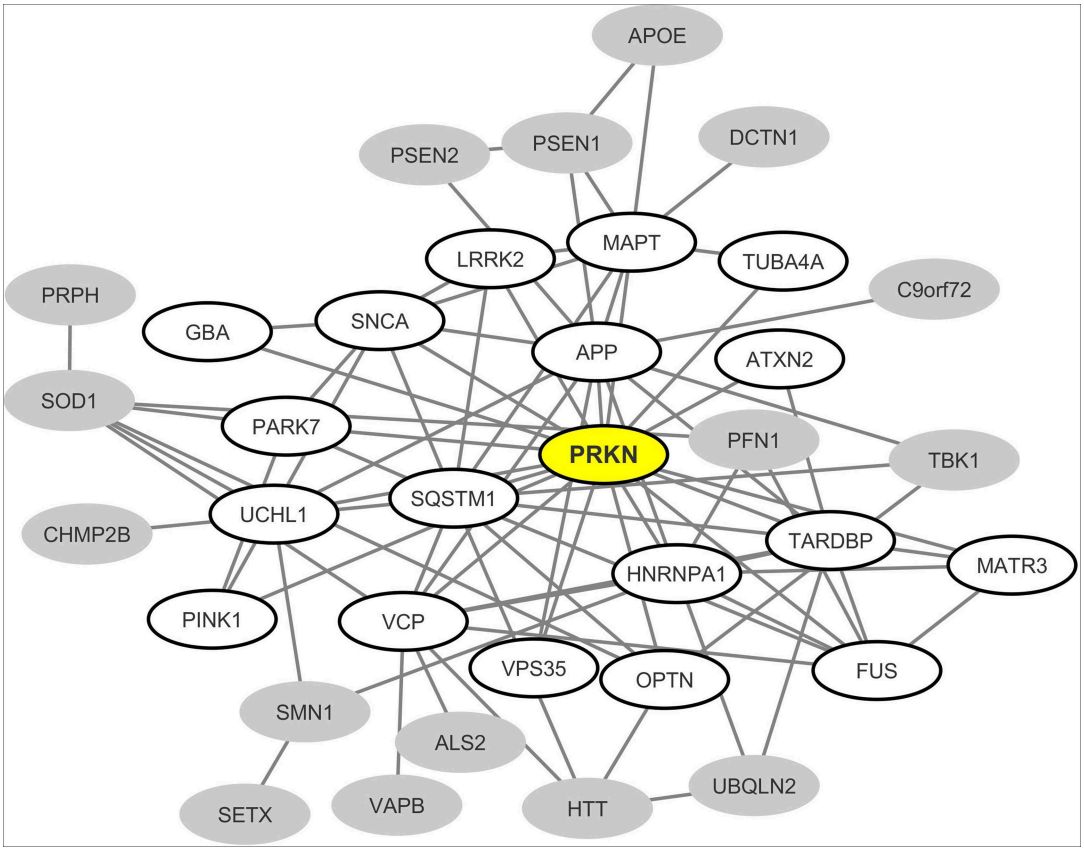

FIGURE 2 | Mapping of the protein-protein interaction network linking common neurodegeneration-associated proteins irrespective of the cell type considered. A survey of the human proteome was performed by querying the protein-protein interaction database BioGRID (38). Each node represents a protein indicated by the corresponding gene symbol and each edge represents an experimentally-demonstrated protein-protein interaction. In this network, the "PRKN" (Parkin) node, highlighted in yellow, exhibits the highest degree (i.e., the highest number of direct interactors). White nodes correspond to the first-shell partners of PRKN. Gray nodes are not direct interactors of PRKN.

a large array of proteins involved in not only PD but also ALS, AD, Huntington's disease and/or FTD.

A Majority of Common Neurodegeneration-Associated Proteins Are Abundantly Expressed in Human B Lymphocytes

We then explored proteomics databases to determine whether neurodegeneration-associated proteins are expressed by APCs under physiological conditions. Our search was restricted to 2 cell lineages which harbor demonstrated antigen-presenting functions: B lymphocytes (46–48) and dendritic cells (DCs) (49–51). Results were combined with those retrieved from proteomics data obtained in 3 independent studies by the mass spectrometry analysis of human B lymphocytes (40) or human dendritic cells (41, 42). Since mass spectrometry is not a sensitive technique, one may consider that detected proteins are abundantly expressed. As shown in **Table 1**, it is striking that 34 (70%) of 48 common neurodegeneration-associated proteins are detectable by mass spectrometry in human B lymphocytes (**Table 1**). In human DCs, 20 (41%) of 48 common neurodegeneration-associated proteins were detected and all of these were also detected in human B lymphocytes. Conversely, 14 common degeneration-associated proteins were reported to be detected in B lymphocytes but not DCs. These include notably apolipoprotein E, amyloid beta A4 protein, presenilin-1 and alpha-synuclein.

Of note, in both human B lymphocytes and DCs, parkin, the *PRKN*-encoded protein, was undetectable as assessed by mass spectrometry. This finding urged us to identify the main hub protein(s) which may interconnect neurodegeneration-associated proteins that are abundantly expressed in human B lymphocytes.

Neurodegeneration-Associated Proteins Expressed by Human B Lymphocytes Form a Unique Interaction Network in Which the Inflammation/Autophagy-Related Protein SQSTM1 Is the Main Hub

From the interaction network depicted in **Figure 2** we only retained nodes corresponding to neurodegeneration-associated genes that are detectable by mass spectrometry in human B lymphocytes. We observed that in this cell type, 24 neurodegeneration-associated proteins are predicted to form a tight interaction network which is centered on the

TABLE 1 | Expression patterns of human neurodegeneration-associated proteins in B-cells vs. dendritic cells.

Gene symbol	Protein ID	Blood-derived B lymphocytes (39)	Blood-derived B lymphocytes (40)	Blood-derived DCs (41)	Cultured MoDCs (42)
APOE	P02649	Detected	detected	0	0
APP	P05067	Detected	0	0	0
ATXN2	Q99700	Detected	Detected	0	0
CHMP2B	Q9UQN3	Detected	Detected	0	Detected
DCTN1	Q14203	Detected	Detected	0	Detected
FIG4	Q92562	Detected	Detected	0	0
FUS	P35637	Detected	Detected	detected	Detected
GBA	P04062	0	Detected	0	Detected
GRN	P28799	Detected	Detected	0	0
HNRNPA1	P09651	Detected	0	0	Detected
HTT	P42858	Detected	Detected	Detected	Detected
LRRK2	Q5S007	Detected	Detected	Detected	0
MATR3	P43243	Detected	Detected	Detected	Detected
OPTN	Q96CV9	Detected	Detected	0	0
PARK7	Q99497	Detected	Detected	Detected	Detected
PFN1	P07737	Detected	Detected	Detected	Detected
PRPH	P41219	Detected	0	0	Detected
PSEN1	P49768	Detected	0	0	0
SETX	Q7Z333	Detected	Detected	0	Detected
SIGMAR1	Q99720	Detected	Detected	0	0
SNCA	P37840	Detected	0	0	0
SOD1	P00441	Detected	Detected	Detected	Detected
SPG11	Q96JI7	Detected	Detected	0	0
SQSTM1	Q13501	Detected	Detected	0	Detected
TARDBP	Q13148	Detected	0	Detected	Detected
TBK1	Q9UHD2	Detected	Detected	0	Detected
TBP	P20226	Detected	Detected	0	0
TRPM7	Q96QT4	0	Detected	0	0
TUBA4A	P68366	Detected	0	0	Detected
UBQLN2	Q9UHD9	Detected	Detected	0	0
UCHL1	P09936	Detected	Detected	0	0
VAPB	O95292	Detected	Detected	Detected	Detected
VCP	P55072	Detected	Detected	Detected	Detected
VPS35	Q96QK1	Detected	Detected	Detected	Detected

The proteomics profiles of human circulating B lymphocytes, circulating dendritic cells (DCs) or cultured monocyte-derived dendritic cells (MoDCs) were retrieved from 4 independent mass spectrometry studies (39–42). We found that 34 from 48 common neurodegeneration-associated proteins were detected by mass spectrometry in at least 1 of the explored studies. Interestingly, 14 common degeneration-associated proteins (highlighted in gray) were reported to be detected in B lymphocytes but not DCs. These include notably apolipoprotein E, amyloid beta A4 protein, presenilin-1 and alpha-synuclein. In contrast, when detected in DCs, common neurodegeneration-associated proteins were constantly detected in B lymphocytes.

inflammation/autophagy-related protein SQSTM1 (**Figure 3**). Again, it should be noticed that the great majority of the protein-protein interactions reported so far between SQSTM1 and other common neurodegeneration-associated proteins were demonstrated via robust low throughput biochemical approaches as detailed in **Data Supplement 1**.

The Protein Network Formed by Neurodegeneration-Associated Proteins Connects With a Set of First Shell Interactors Among Which the B-Cell Inflammation/Autophagy-Related Protein TRAF6 Is the Main Hub

To extend these data, we then retrieved from the "BioGrid" database the currently known and experimentally-demonstrated interactors of common neurodegeneration-associated proteins, irrespective of their levels of expression in human B lymphocytes. From this list, we extracted candidate hub proteins interacting with more than 10 neurodegeneration-associated proteins. For each of these candidate hubs, total lists of protein interactors were then retrieved from BioGrid and a Fisher's exact test was applied so to determine if neurodegeneration-associated proteins were actually significantly enriched in each established list. By this mean, we were able to assign an enrichment factor and an associated *p*-value to each candidate hub. This approach allowed us to identify 20 hub proteins (**Table 2**) with which common neurodegeneration-associated proteins are specifically connected. Among these 20 hub proteins, 10 are expressed at the protein level as assessed via the "Human Proteome Map" database (39). Moreover, from the 10 hub proteins exhibiting the most significant and highest enrichment factors, 8 are abundantly expressed by human B lymphocytes. These include: (i) the heat shock proteins HSPA8 (also named HSC70), HSPA4 (also

TABLE 2 | Main hub partners of common neurodegeneration-associated proteins.

Gene symbol	Protein ID	Protein partners	ND-associated protein partners	Enrichment factor	Adjusted *p*-value
TRAF6	Q9Y4K3	315	17	28.40	1.03E-17
PRKN	O60260	454	18	20.86	2.77E-16
SQSTM1	Q13501	309	13	22.14	8.25E-13
HSPA4	P34932	363	13	17.90	1.58E-10
HSPA8	P11142	708	16	11.29	8.20E-10
YWHAZ	P63104	412	12	14.56	9.38E-9
HSP90AA1	P07900	839	15	8.93	6.65E-08
YWHAQ	P27348	476	11	11.55	4.74E-07
CYLD	Q9NQC7	620	12	9.67	8.15E-07
CTNNB1	P35222	616	11	8.92	6.00E-06
UBC	P0CG48	1050	14	6.66	6.54E-06
TP53	P04637	1062	14	6.59	7.46E-06
CUL7	Q14999	658	11	8.35	1.13E-05
EGFR	P00533	1224	14	5.71	3.73E-05
RNF4	P78317	1251	14	5.59	4.75E-05
MCM2	P49736	940	11	5.85	3.09E-04
BRCA1	P38398	962	11	5.71	3.80E-04
NTRK1	P04629	1948	16	4.10	4.54E-04
CUL3	Q13618	1227	13	5.29	1.75E-04
ESR2	Q92731	2249	13	2.89	4.67E-02

A survey of the human proteome was performed by querying the protein-protein interaction database BioGRID (38). Candidate hub proteins interacting with more than 10 common neurodegeneration-associated proteins were retrieved. The lists of partners currently demonstrated for each of these candidate hubs were then retrieved. For each of these lists, a factor of enrichment in neurodegeneration-associated proteins was established along with an associated Fisher's exact test p-value, as described in the Materials and Methods section. From left to right, gene symbols and corresponding protein IDs of the identified hub proteins are shown in the first and second column, the associated total numbers of partners and total numbers of neurodegeneration (ND)-associated protein interactors are shown in the third and fourth column. The corresponding enrichment factors and adjusted p-values are shown in the last two columns. Enrichment factors and statistical analyses were performed on data retrieved from the 3.5.175 release of BioGrid or, for PRKN, SQSTM1 and TRAF6, from the 3.5.177 release of BioGrid. Proteins that are detectable by mass spectrometry in human B lymphocytes, according to the "Human Proteome Map" database (39), are highlighted in gray.

TABLE 3 | Enrichment analysis of TRAF6 partners list.

Term	Adjusted *p*-value
Disease enrichment	
Cancer	5.09E-7
Frontotemporal dementia	2.69E-6
Hypohidrotic ectodermal dysplasia	2.75E-6
Toxic encephalopathy	3.20E-6
Mulibrey nanism	3.21E-6
Neurodegenerative disease	3.45E-6
Arthritis	4.14E-6
Influenza	8.77E-6
Pick's disease	1.93E-4
Leishmaniasis	3.72E-4

A survey of the human proteome was performed by querying the protein-protein interaction database BioGRID (38). The list of currently known and experimentally-demonstrated TRAF6 partners was retrieved. From this list of interactors, an enrichment analysis with the "JENSEN Disease" text-mining webtool (37) embedded in the "Enrichr" analysis platform (36) was then performed. Shown are the disease-related terms exhibiting the 10 most statistically significant enrichments. Terms relating with neurodegeneration are highlighted in gray.

named HSP70) and HSP90AA1 (also named HSP90) which are all involved in both antigen presentation by MHC class II molecules (52–54) and the modulation of T-cell responses (55–57) and (ii) YWHAZ and YWHAQ proteins also known as 14-3-3 protein zeta and theta respectively, which bind MHC class II molecules (58, 59) and are implicated in various neurodegenerative diseases (60–62). Finally, unexpectedly, the most significant hub we identified was TRAF6 (TNF receptor associated factor 6), an inflammation/autoimmunity-related molecule (63, 64) playing a major role in the control of B-cell activation (65, 66). Indeed, the whole list of TRAF6 interactors comprises 17 neurodegeneration-associated proteins i.e., 5.3%, which corresponds to an enrichment factor of 27.68 and an adjusted *p*-value of $1.03E^{-17}$ (Fisher's exact test). The robustness of the biochemical techniques which were used to identify TRAF6 neurodegeneration-associated protein partners was checked as detailed in **Data Supplement 1**.

However, one may argue that the list of currently-known TRAF6 partners might be enriched in not only neurodegeneration-associated proteins but also many other sets of proteins which do not relate with neurodegeneration. To address this issue, we performed on the whole list of TRAF6 partners a non *a priori* enrichment analysis using the "JENSEN Disease" text-mining webtool (37) embedded in the "Enrichr" analysis platform (36). Results shown in **Table 3** indicate that the 10 disease-related terms with which TRAF6 partners are the most significantly associated comprise the terms "Frontotemporal dementia," "Neurodegenerative disease" and "Pick's disease." This finding points to a specific link between TRAF6 and neurodegeneration.

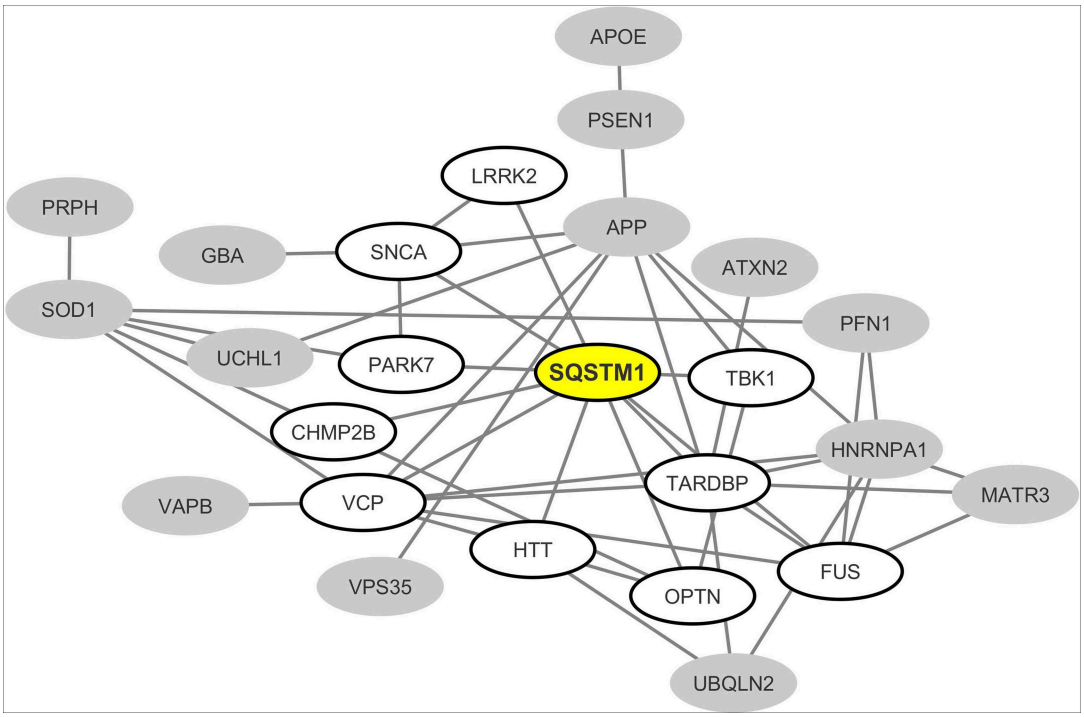

FIGURE 3 | Mapping of the protein-protein interaction network linking common neurodegeneration-associated proteins which are expressed in human B lymphocytes. A survey of the human proteome was performed by querying the protein-protein interaction database BioGRID (38). Each node represents a protein indicated by the corresponding gene symbol and each edge represents an experimentally-demonstrated protein-protein interaction. In this network, the "SQSTM1" (Sequestosome-1) node, highlighted in yellow, exhibits the highest degree (i.e., the highest number of direct interactors). White nodes correspond to the first-shell partners of SQSTM1 in human B lymphocytes. Gray nodes are expressed by B lymphocytes but are not direct interactors of SQSTM1.

As a control and to further establish the specificity of our findings, we assessed whether similar results would be obtained from a list of common demyelination-associated proteins i.e., proteins commonly considered as candidate autoantigens in multiple sclerosis (67). We found that neither TRAF6 nor the other hubs linking common neurodegeneration-associated proteins exhibited lists of protein partners which were significantly enriched in common demyelination-associated proteins (**Data Supplement 2**).

From these results we then built and visualized a B-cell protein-protein interaction network encompassing the most significant hub proteins and their neurodegeneration-associated interactors expressed in B lymphocytes (**Figure 4**). We observed that from the 32 neurodegeneration-associated proteins expressed by B lymphocytes, 22 (68%) are first shell interactors of HSPA4, HSPA8, TRAF6, or SQSTM1. These results unravel a yet unknown function of these molecules as major hub proteins connecting in B lymphocytes a large array of proteins involved in PD, ALS, AD, Huntington's disease and/or FTD.

Common Neurodegeneration-Associated Proteins Provide a Source of Endogenous MHC Class II-Binding Peptides in Human B Lymphocytes

While peptides presented by MHC class II molecules are classically generated by the proteolysis of phagocytized exogenous antigens, the presentation of endogenous peptides by MHC class II molecules is an alternate pathway which has been robustly documented (68–71). We thus surveyed the Immune Epitope DataBase (IEDB-AR) (43) to assess whether peptides derived from neurodegeneration-associated proteins had been previously identified as binding MHC class II molecules in immunopeptidome studies which used human cells of the B-cell lineage as a source of endogenous peptides. Of note, we excluded studies assessing the MHC binding of exogenously-provided specific peptides and retained only works relying on the systematic mass spectrometry-based identification of peptides eluted from MHC class II molecules. In addition, we excluded experiments in which immunization or stimulation protocols were applied to B lymphocytes. On this basis, we retained 19 studies (**Data Supplement 3**) which were performed on cells of the B-cell lineage including predominantly Epstein-Barr virus (EBV)-transformed B lymphocytes. When screening these studies, we found that 23 out of 48 common neurodegeneration-associated proteins were reported to provide endogenous MHC class II-binding peptides in B lymphocytes (**Table 4** and **Data Supplement 3**). The most frequently identified neurodegeneration-associated parent proteins are abundantly expressed in B lymphocytes and comprise notably the proteins encoded by: (i) the AD-related genes *APP* and *PSEN1*, (ii) the ALS/FTD-related genes *PFN1*, *SQSTM1*, *GRN*, *SOD1,* and *VCP* and (iii) the PD-related proteins *PARK7* and *GBA* (**Figure 1**).

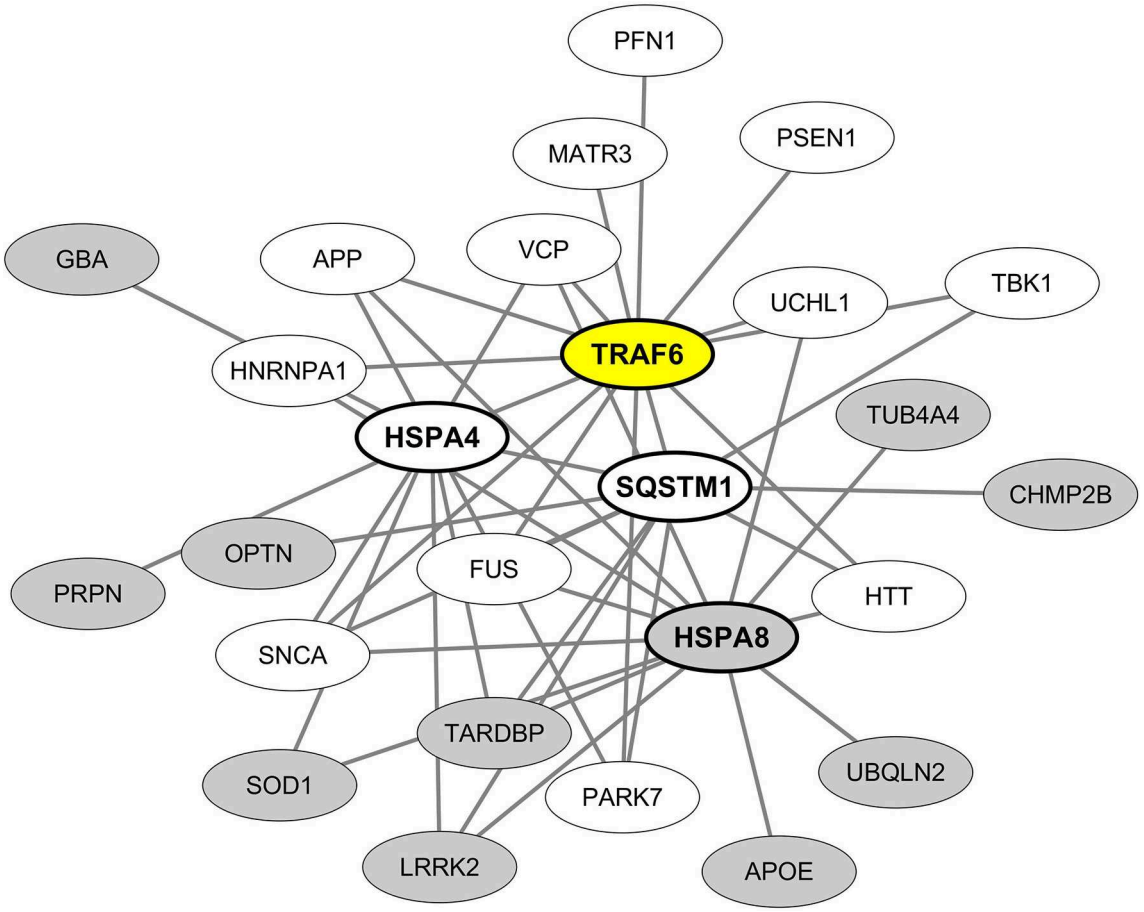

FIGURE 4 | Mapping of the protein-protein interaction network linking common neurodegeneration-associated proteins expressed in B lymphocytes and their hub protein partners. A survey of the human proteome was performed by querying the protein-protein interaction database BioGRID (38). Only proteins expressed by human B lymphocytes according to the "Human Proteome Map" database (39) were taken into account. Each node represents a protein indicated by its corresponding gene symbol. The 4 hub proteins expressed by B lymphocytes and whose partners exhibit the most significant and highest enrichment factors in neurodegeneration-associated proteins are indicated in bold characters. White nodes correspond to neurodegeneration-associated proteins expressed by B lymphocytes and interacting with TRAF6. Gray nodes correspond to common neurodegeneration-associated proteins expressed by B lymphocytes but not reported to interact with TRAF6.

Hub Molecules Linking Common Neurodegeneration-Associated Proteins Provide a Source of Endogenous MHC Class II-Binding Peptides in Human B Lymphocytes

From the 19 relevant B-cell studies we retained on IEDB-AR, we also attempted to determine whether the hub molecules we identified as linking neurodegeneration-associated proteins in B lymphocytes (**Table 2**) were, in parallel, reported to provide endogenous ligands for MHC class II molecules in B lymphocytes. We found that from 10 candidate hubs abundantly expressed by B lymphocytes, 8 were reported to provide endogenous ligands for MHC class II molecules in human B lymphocytes (**Table 5** and **Data Supplement 3**).

Among these 8 hubs, HSPA8 was the parent protein being the most frequently identified as providing endogenous peptides which bind MHC class II molecules in human B lymphocytes (**Table 5**).

In Human B Lymphocytes, the List of Parent Proteins From Which Derive Endogenous MHC Class II-Binding Peptides Is Specifically Enriched in Neurodegeneration-Associated Proteins

The whole list of parent proteins identified as providing MHC class II-binding endogenous peptides in B lymphocytes was retrieved from IEDB-AR and an enrichment analysis was performed to determine whether such a list was significantly enriched in neurodegeneration-associated proteins. We found that genes coding for neurodegeneration-associated proteins encompassed 0.62% (22 out of 3523) of the whole genes coding for such parent proteins (**Data Supplement 4**), which

TABLE 4 | List of neurodegeneration-associated proteins from which derive MHC class II-binding endogenous peptides in the human B-cell lineage.

Neurodegeneration-associated proteins	Reported MHC class II restrictions
Amyloid beta A4 protein (*APP*)	HLA class II, HLA-DQ, HLA-DR, HLA-DR3, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2, HLA-DRB1*15:01 (HLA-DR15), HLA-DRB5*01:01 (HLA-DR51), HLA-DRB1*04:05 (HLA-DR4), HLA-DQA1*05:01/DQB1*02:01 (HLA-DQ2.5)
Sequestosome-1 (*SQSTM1*)	HLA class II, HLA-DR, HLA-DQ, HLA-DR1, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2, HLA-DR15/DR51, HLA-DQA1*05:01/DQB1*02:01 (HLA-DQ2.5)
Profilin-1 (*PFN1*)	HLA class II, HLA-DR, HLA-DQ, HLA-DR1, HLA-DR3, HLA-DRB1*04:05 (HLA-DR4), HLA-DR15/DR51
Superoxide dismutase [Cu-Zn] (*SOD1*)	HLA class II, HLA-DR, HLA-DR1, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2
Transitional endoplasmic reticulum ATPase (*VCP*)	HLA class II, HLA-DR, HLA-DR1, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2
Lysosomal acid glucosylceramidase (*GBA*)	HLA class II, HLA-DR, HLA-DQ, HLA-DR1, HLA-DR15, HLA-DRB1*04:05 (HLA-DR4)
Presenilin-1 (*PSEN1*)	HLA class II, HLA-DQ, HLA-DQ2.2, HLA-DR15/DR51, HLA-DRB1*04:05 (HLA-DR4)
Ubiquilin-2 (*UBQLN2*)	HLA class II, HLA-DR1, HLA-DQ7.5, HLA-DQ2.2, HLA-DRB1*04:05 (HLA-DR4)
Major prion protein (*PRNP*)	HLA-DQ, HLA-DR, HLA-DR1, HLA-DQ7.5, HLA-DQ2.2
Vacuolar protein sorting-associated protein 35 (*VPS35*)	HLA class II, HLA-DR, HLA-DR15/DR51, HLA-DQA1*05:01/DQB1*02:01 (HLA-DQ2.5)
Optineurin (*OPTN*)	HLA-DR1, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2
Protein deglycase DJ-1 (*PARK7*)	HLA class II, HLA-DR1, HLA-DRB1*04:01 (HLA-DR4), HLA-DRB1*04:05 (HLA-DR4)
Progranulin (*GRN*)	HLA class II, HLA-DQ, HLA-DQ7.5
Spatacsin (*SPG11*)	HLA-DQ7.5, HLA-DQ2.2
Ubiquitin carboxyl-terminal hydrolase isozyme L1 (*UCHL1*)	HLA class II, HLA-DR
RNA-binding protein FUS (*FUS*)	HLA class II
Probable cation-transporting ATPase 13A2 (*ATP13A2*)	HLA-DR
Heterogeneous nuclear ribonucleoprotein A1 (*HNRNPA1*)	HLA class II
Charged multivesicular body protein 2b (*CHMP2B*)	HLA-DQ7.5
Huntingtin (*HTT*)	HLA-DR
Serine/threonine-protein kinase TBK1 (*TBK1*)	HLA-DR15/DR51
Presenilin-2 (*PSEN2*)	HLA-DR15/DR51
Microtubule-associated protein tau (*MAPT*)	HLA-DR15/DR51

The "IEDB-AR" immune epitope database (43) was screened in order to retain only publications reporting on the systematic mass spectrometry identification of endogenous peptides binding MHC-class II molecules in the human B-cell lineage. Only results obtained on human cells of the B-cell lineage in the absence of immunization or stimulation protocols were taken into account. The retrieved list of parent proteins was then crossed with the list of 48 common neurodegeneration-associated proteins we established. The protein names and gene symbols are shown in the left column; the corresponding reported MHC class II restrictions of derived peptides are shown in the right column. When needed, aliases frequently used in the HLA class II genotype/serotype nomenclature are indicated in brackets.

corresponds to an enrichment of 2.58 (p-value = 0.0006, Fisher's exact test) when considering the whole number of human protein-coding genes as roughly 20 000 (72). To confirm these results and assess their level of specificity, we used the "JENSEN Disease" text-mining webtool (37) and performed an unsupervised enrichment analysis from the whole list of genes coding for parent proteins previously identified as providing MHC class II-binding peptides in human B lymphocytes (**Data Supplement 4**). From this list of 3522 genes, 96 (2.72%) were annotated with the term "Neurodegenerative disease" (**Data Supplement 4**) which corresponds to the second most significant enrichment, after the term "Arthritis" (**Table 6**).

Since the HLA-DRB1 1501 allele (corresponding to the HLA-DR15 serotype) was recently identified as a risk factor for sporadic forms of late onset AD (14), we retrieved the whole list of parent proteins (and corresponding coding genes) which, in human B lymphocytes, were previously reported to provide peptides that bind HLA-DRB1 1501-encoded MHC class II molecules. Importantly, such a list was significantly enriched in neurodegeneration-associated proteins (enrichment factor: 5.31; p-value = 0.0001, Fisher exact test). Moreover, when this list of parent proteins was submitted to an unsupervised enrichment analysis, the term "Neurodegenerative disease" was found to reach the highest level of statistical significance (**Table 7**).

Finally, based on the IEDB-AR survey we performed, HLA-DRB1 1501-encoded MHC class II molecules are the only HLA class II molecules which, in human B lymphocytes, were reported to bind endogenous peptides deriving from microtubule-associated protein tau, presenilin-2 or serine/threonine-protein kinase TBK1 (**Table 4** and **Data Supplement 3**).

DISCUSSION

In the present work, we mined large publically-available databases to provide experiment-based evidence of a link

TABLE 5 | List of the main hub partners linking neurodegeneration-associated proteins and giving rise to MHC class II-binding endogenous peptides in the human B-cell lineage.

Hub molecules linking common neurodegeneration-associated proteins	Reported MHC class II restriction
Heat shock cognate 71 kDa protein (*HSPA8*)	HLA class II, HLA-DR, HLA-DQ, HLA-DR1, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2, HLA-DR3, HLA-DRB1*15:01 (HLA-DR15), HLA-DR15/DR51, HLA-DQA1*05:01/DQB1*02:01 (HLA-DQ2.5), HLA-DRB1*04:01 (HLA-DR4), HLA-DRB1*04:05 (HLA-DR4)
Sequestosome-1 (*SQSTM1*)	HLA class II, HLA-DR, HLA-DQ, HLA-DR1, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2, HLA-DR15/DR51, HLA-DQA1*05:01/DQB1*02:01 (HLA-DQ2.5)
Heat shock protein HSP 90-alpha (*HSP90AA1*)	HLA class II, HLA-DR, HLA-DQ, HLA-DQ7.5, HLA-DQ2.5, HLA-DQ2.2, HLA-DRB1*04:05 (HLA-DR4), HLA-DRB1*15:01 (HLA-DR15) HLA-DQA1*05:01/DQB1*02:01 (HLA-DQ2.5)
14-3-3 protein zeta/delta (*YWHAZ*)	HLA class II, HLA-DR, HLA-DQ, HLA-DR1, HLA-DRB1*04:05 (HLA-DR4)
4-3-3 protein theta (YWHAQ)	HLA class II, HLA-DQ, HLA-DRB1*04:05 (HLA-DR4)
Heat shock 70 kDa protein 4 (*HSPA4*)	HLA-DR, HLA-DR3
Ubiquitin carboxyl-terminal hydrolase CYLD (*CYLD*)	HLA-DRB1*04:05 (HLA-DR4)
TNF receptor-associated factor 6 (*TRAF6*)	HLA-DR1

The "IEDB-AR" immune epitope database (43) was screened in order to retain only publications reporting on the systematic mass spectrometry identification of endogenous peptides binding MHC-class II molecules in the human B-cell lineage. Only results obtained in the absence of immunization or stimulation protocols were taken into account. The retrieved list of parent proteins and corresponding reported MHC class II restriction of derived peptides was then crossed with the list of 11 hub molecules being abundantly expressed by human B lymphocytes and linking common neurodegeneration-associated. The protein names and corresponding gene symbols are shown in the left column; the corresponding reported MHC class II restrictions of derived peptides are shown in the right column. When needed, aliases frequently used in the HLA class II genotype/serotype nomenclature are indicated in brackets.

TABLE 6 | Enrichment analysis of the whole list of parent proteins giving rise to MHC class II-binding endogenous peptides in the human B-cell lineage.

Term	Adjusted *p*-value
Disease enrichment	
Arthritis	3.94E-8
Neurodegenerative disease	1.80E-7
Lupus erythematosus	4.92E-7
Tetanus	2.07E-5
Human immunodeficiency virus infectious disease	4.80E-5
Diamond-Blackfan anemia	7.10E-5
Coronary artery disease	1.57E-4
Fabry disease	2.08E-4
Inclusion-cell disease	2.16E-4
Neuropathy	1.97E-4

The "IEDB-AR" immune epitope database (43) was screened in order to retain only publications reporting on the systematic mass spectrometry identification of endogenous peptides binding MHC-class II molecules in the human B-cell lineage. Only results obtained in the absence of immunization or stimulation protocols were taken into account. The parent proteins from which derive the identified bound peptides were retrieved and the list of corresponding coding genes was submitted to an enrichment analysis with the "JENSEN Disease" text-mining webtool (37) embedded in the "Enrichr" analysis platform (36). The 10 most significant terms associated with this list of genes are shown. The second most significant enrichment is observed for the term "Neurodegenerative disease" (term and corresponding adjusted p-value highlighted in gray).

TABLE 7 | Enrichment analysis of the whole list of parent proteins giving rise to endogenous peptides binding HLA-DRB1 1501-encoded MHC class II molecules in the human B-cell lineage.

Term	Adjusted *p*-value
Disease enrichment	
Neurodegenerative disease	7.8E-6
Influenza	2.1E-4
Allergic hypersensitivity disease	2.8E-4
Lupus erythematous	1.2E-3
inclusion-cell disease	1.6E-3
Gangliosidosis	1.6E-3
Diamond-Blackfan anemia	1.8E-3
Tetanus	2.0E-3
Lysosomal storage disease	2.3E-3
Dementia	3.1E-3

The "IEDB-AR" immune epitope database (43) was screened in order to retain only publications reporting on the systematic mass spectrometry identification of endogenous peptides binding MHC-class II molecules in the human B-cell lineage. Only results obtained in the absence of immunization or stimulation protocols were taken into account. The parent proteins from which derive the identified bound peptides were retrieved and the list of corresponding coding genes was submitted to an enrichment analysis with the "JENSEN Disease" text-mining webtool (37) embedded in the "Enrichr" analysis platform (36). Shown are the disease-related terms exhibiting the 10 most statistically significant enrichments. Terms relating with neurodegeneration are highlighted in gray.

between neurodegeneration and autoimmunity. Using a systems biology approach we report that a large range of common neurodegeneration-associated proteins: (i) are expressed by human B lymphocytes under physiological conditions, (ii) form a comprehensive and functionally-relevant protein-protein interaction network and (iii) provide endogenous peptides which bind MHC class-II molecules in human B lymphocytes. Patients suffering from neurodegenerative conditions exhibit T cell- and/or antibody-mediated responses directed against major neurodegeneration-associated proteins such as amyloid beta A4 protein, alpha-synuclein and tau protein (4–6, 73). However, naturally-occurring antibodies against amyloid-beta (74), alpha-synuclein (75–77) and tau protein (73, 77, 78) were also demonstrated in cohorts of healthy subjects. Similarly, apart from any pathological context, robust T-cell responses against peptides deriving from tau protein were recently demonstrated to widely occur in the general population (79). These findings suggest that the autoimmune processes described in patients with neurodegenerative conditions might be shaped by pre-existing

physiological autoimmune responses directed against common neurodegeneration-associated proteins. It is worth noting that, while physiological autoimmunity was firmly demonstrated nearly 50 years ago (80–82), the intimate nature of the links bridging physiological autoimmunity to its pathological counterpart is still matter of debate. Numerous functions have been assigned to physiological autoimmunity (83, 84), including, more recently, a support to cognition (85–90). In line with these findings, our data indicate that a specific set of brain antigens expressed by B lymphocytes and involved in neurodegenerative diseases might prime a neuroprotective and, possibly, cognition-promoting T-cell response under physiological conditions. Of note, B lymphocytes are now recognized as professional APCs (46–48) and, most interestingly, memory B lymphocytes were demonstrated to trigger the activation of autoreactive T-cells in an MHC-class II-dependent manner (91). Indeed, the autophagy of cytosolic and nuclear proteins in B lymphocytes provide a continuous source of endogenous MHC class-II ligands (92) and such autophagosome-derived peptides induce the proliferation of autologous T-cells under *in vitro* conditions (93). However, that neurodegeneration-associated proteins provide MHC class II-binding endogenous peptides in B lymphocytes neither prove that T-cells are actually primed against such peptides *in vivo* nor that peptides deriving from misfolded neurodegeneration-associated proteins are presented by B lymphocytes under neurodegenerative conditions. Furthermore, even if it was actually the case, the phenotype of autoreactive T-cells generated via such a mechanism would need to be explored. More generally, one has to keep in mind that no consensus has been currently reached regarding the phenotype of physiological autoreactive T-cells. Thus, both autoreactive Tregs and autoreactive pro-inflammatory T-cells belong to the physiological T-cell repertoire and were both found to exert neuroprotective effects (94–97).

Several genes involved in familial forms of neurodegenerative disorders exert key functions in the autophagy pathway. These notably comprise PRKN, PINK1, and SQSTM1 (98–101). In the recent years, major works provided evidence that, in neurons and immune cells, functional defects in such genes hamper mitophagy (a specialized form of autophagy), stimulate the inflammasome pathway and foster the presentation of mitochondrial antigens by MHC class I molecules (33, 102, 103). However, these findings do not render account for the existence of HLA class II-restricted T-cell responses against neurodegeneration-associated proteins. Moreover, antigens targeted by autoimmunity during neurodegenerative conditions are far from deriving only from the mitochondrial compartment. In this regard, our work suggests that in B lymphocytes, the inflammation/autophagy-related molecules SQSTM1 and TRAF6 are crucially involved in the presentation of neurodegeneration-associated antigens by MHC class II molecules. However, while our data mining approach was restricted to the retrieval of experimentally-demonstrated protein-protein interactions, whether such interactions actually occur in human B lymphocytes remains to be ascertained. This issue clearly requires to be addressed in future studies.

The mining of previously published mass spectrometry analyses performed on MHC class II-eluted peptides showed that, in human B lymphocytes, several neurodegeneration-associated proteins provide endogenous peptides which bind a large range of MHC class II alleles. This is notably the case for amyloid beta A4 protein, sequestosome-1 and profilin-1. Similarly, peptides deriving from HSPA8, a hub molecule which links a high number of neurodegeneration-associated proteins, bind multiple MHC class II alleles. Our observations suggest that these 4 molecules are likely to elicit immune responses in a large range of the human population. Determining whether or not such antigens trigger cognition-promoting autoimmunity is a potentially important issue. On another hand, in human B lymphocytes, several neurodegeneration-associated antigens appear to provide endogenous peptides harboring an allele-specific MHC class II restriction in B lymphocytes. For e.g., endogenous peptides deriving from microtubule-associated protein tau, PSEN2 and Serine/threonine-protein kinase TBK1 were exclusively reported to bind HLA-DRB1 1501-encoded MHC class II molecules under the experimental conditions described above. Since the HLA-DRB1 1501 allele is a susceptibility gene for late onset AD (14), this may prove to be of interest in the context of AD pathophysiology. In particular, although a widespread T-cell reactivity against Tau peptides was recently demonstrated in the general human population (79), this finding indicates that individuals bearing the HLA-DRB1 1501 allele may exhibit qualitative and/or quantitative specificities regarding their anti-Tau T-cell responses. Finally, it should be noticed that in subjects bearing the HLA-DRB1 1501 allele, B lymphocytes were shown to express membrane-anchored HLA-DRB1 1501-encoded MHC molecules (104) and to efficiently present autoantigens to CD4 T-cells via such MHC class II molecules (91).

We previously proposed that the genetic polymorphism of the HLA-DRB1 locus, which, among primates, is extremely high in the human species (105), might allow the allele-specific presentation of distinct sets of "brain superautoantigens" (106, 107) leading, in turn, to the development and maintenance of distinct sets of cognition-promoting T-cells. The present work indicates that common neurodegeneration-associated proteins might represent an important share of brain superautoantigens. Interestingly, recent magnetic resonance imaging studies reported that, in healthy subjects, specific HLA-DR alleles correlate with the volume ranges of specific brain structures (108, 109). Future studies should be designed to determine whether HLA-DR polymorphism might match both brain structural features and the diversity of T-cell responses against common neurodegeneration-associated proteins. Finally, in the human B-cell lineage, MHC class II-binding endogenous peptides are highly significantly and specifically enriched in peptides deriving from common neurodegeneration-associated proteins. This result raises the intriguing possibility that a main function of physiological autoimmunity could be to control the blood-circulating levels of aggregated forms of amyloid beta A4 protein, alpha-synuclein, tau protein and possibly other neurodegeneration-associated proteins.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://thebiogrid.org/, https://haemosphere.org/, http://www.humanproteomemap.org/, https://www.iedb.org/.

AUTHOR CONTRIBUTIONS

SN performed the bioinformatics analyses and wrote the paper. MG and LP performed quality controls of bioinformatics analyses and wrote the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.02704/full#supplementary-material

REFERENCES

1. Ilieva H, Polymenidou M, Cleveland DW. Non–cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. *J Cell Biol*. (2009) 187:761–72. doi: 10.1083/jcb.200908164
2. Scekic-Zahirovic J, El Oussini H, Mersmann S, Drenner K, Wagner M, Sun Y, et al. Motor neuron intrinsic and extrinsic mechanisms contribute to the pathogenesis of FUS-associated amyotrophic lateral sclerosis. *Acta Neuropathol*. (2017) 133:887–906. doi: 10.1007/s00401-017-1687-9
3. Boillee S, Yamanaka K, Lobsiger CS, Copeland NG, Jenkins NA, Kassiotis G, et al. Onset and progression in inherited ALS determined by motor neurons and microglia. *Science*. (2006) 312:1389–92. doi: 10.1126/science.1123511
4. Monsonego A, Zota V, Karni A, Krieger JI, Bar-Or A, Bitan G, et al. Increased T cell reactivity to amyloid β protein in older humans and patients with Alzheimer disease. *J Clin Invest*. (2003) 112:415–22. doi: 10.1172/JCI200318104
5. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, et al. T cells from patients with Parkinson's disease recognize α-synuclein peptides. *Nature*. (2017) 546:656–61. doi: 10.1038/nature22815
6. Garretti F, Agalliu D, Lindestam Arlehamn CS, Sette A, Sulzer D. Autoimmunity in Parkinson's Disease: the role of α-synuclein-specific T cells. *Front Immunol*. (2019) 10:303. doi: 10.3389/fimmu.2019.00303
7. Benner EJ, Banerjee R, Reynolds AD, Sherman S, Pisarev VM, Tsiperson V, et al. Nitrated α-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. *PLoS ONE*. (2008) 3:e1376. doi: 10.1371/journal.pone.0001376
8. Brochard V, Combadière B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, et al. Infiltration of CD4+ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. *J Clin Invest*. (2008) 119:182–92. doi: 10.1172/JCI36470
9. Laurent C, Dorothée G, Hunot S, Martin E, Monnet Y, Duchamp M, et al. Hippocampal T cell infiltration promotes neuroinflammation and cognitive decline in a mouse model of tauopathy. *Brain*. (2017) 140:184–200. doi: 10.1093/brain/aww270
10. Sheean RK, McKay FC, Cretney E, Bye CR, Perera ND, Tomas D, et al. Association of regulatory T-cell expansion with progression of amyotrophic lateral sclerosis. *JAMA Neurol*. (2018) 75:681. doi: 10.1001/jamaneurol.2018.0035
11. Beers DR, Zhao W, Appel SH. The role of regulatory T lymphocytes in amyotrophic lateral sclerosis. *JAMA Neurol*. (2018) 75:656. doi: 10.1001/jamaneurol.2018.0043
12. Coque E, Salsac C, Espinosa-Carrasco G, Varga B, Degauque N, Cadoux M, et al. Cytotoxic CD8 $^+$ T lymphocytes expressing ALS-causing SOD1 mutant selectively trigger death of spinal motoneurons. *Proc Natl Acad Sci U.S.A*. (2019) 116:2312–7. doi: 10.1073/pnas.1815961116
13. Niemelä V, Burman J, Blennow K, Zetterberg H, Larsson A, Sundblom J. Cerebrospinal fluid sCD27 levels indicate active T cell-mediated inflammation in premanifest Huntington's disease. *PLoS ONE*. (2018) 13:e0193492. doi: 10.1371/journal.pone.0193492
14. Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. *Nat Genet*. (2019) 51:414–30. doi: 10.1038/s41588-019-0358-2
15. Hollenbach JA, Norman PJ, Creary LE, Damotte V, Montero-Martin G, Caillier S, et al. A specific amino acid motif of *HLA-DRB1* mediates risk and interacts with smoking history in Parkinson's disease. *Proc Natl Acad Sci USA*. (2019) 116:7419–24. doi: 10.1073/pnas.1821778116
16. Witoelar A, Jansen IE, Wang Y, Desikan RS, Gibbs JR, Blauwendraat C, et al. Genome-wide pleiotropy between Parkinson Disease and Autoimmune Diseases. *JAMA Neurol*. (2017) 74:780. doi: 10.1001/jamaneurol.2017.0469
17. Broce I, Karch CM, Wen N, Fan CC, Wang Y, Hong Tan C, et al. Immune-related genetic enrichment in frontotemporal dementia: an analysis of genome-wide association studies. *PLOS Med*. (2018) 15:e1002487. doi: 10.1371/journal.pmed.1002487
18. Pottier C, Ren Y, Perkerson RB, Baker M, Jenkins GD, van Blitterswijk M, et al. Genome-wide analyses as part of the international FTLD-TDP whole-genome sequencing consortium reveals novel disease risk factors and increases support for immune dysfunction in FTLD. *Acta Neuropathol*. (2019) 137:879–99. doi: 10.1007/s00401-019-01962-9
19. Raj T, Rothamel K, Mostafavi S, Ye C, Lee MN, Replogle JM, et al. Polarization of the effects of autoimmune and neurodegenerative risk alleles in leukocytes. *Science*. (2014) 344:519–23. doi: 10.1126/science.1249547
20. Matheoud D, Sugiura A, Bellemare-Pelletier A, Laplante A, Rondeau C, Chemali M, et al. Parkinson's Disease-related proteins PINK1 and parkin repress mitochondrial antigen presentation. *Cell*. (2016) 166:314–27. doi: 10.1016/j.cell.2016.05.039
21. Lall D, Baloh RH. Microglia and C9orf72 in neuroinflammation and ALS and frontotemporal dementia. *J Clin Invest*. (2017) 127:3250–8. doi: 10.1172/JCI90607
22. ORourke JG, Bogdanik L, Yanez A, Lall D, Wolf AJ, Muhammad AKMG, et al. C9orf72 is required for proper macrophage and microglial function in mice. *Science*.(2016) 351:1324–9. doi: 10.1126/science.aaf1064
23. Nataf S, Pays L. Gene co-expression analysis unravels a link between C9orf72 and RNA metabolism in myeloid cells. *Acta Neuropathol Commun*. (2015) 3:64. doi: 10.1186/s40478-015-0242-y
24. Fava VM, Manry J, Cobat A, Orlova M, Van Thuc N, Ba NN, et al. A missense LRRK2 variant is a risk factor for excessive inflammatory responses in leprosy. *PLoS Negl Trop Dis*. (2016) 10:e0004412. doi: 10.1371/journal.pntd.0004412
25. Wong AYW, Oikonomou V, Paolicelli G, De Luca A, Pariano M, Fric J, et al. Leucine-Rich Repeat Kinase 2 Controls the Ca2+/Nuclear Factor of Activated T Cells/IL-2 pathway during aspergillus non-canonical autophagy in dendritic cells. *Front Immunol*. (2018) 9:210. doi: 10.3389/fimmu.2018.00210
26. Härtlova A, Herbst S, Peltier J, Rodgers A, Bilkei-Gorzo O, Fearns A, et al. LRRK2 is a negative regulator of *Mycobacterium tuberculosis* phagosome maturation in macrophages. *EMBO J*. (2018) 37:e98694. doi: 10.15252/embj.201798694

27. Kozina E, Sadasivan S, Jiao Y, Dou Y, Ma Z, Tan H, et al. Mutant LRRK2 mediates peripheral and central immune responses leading to neurodegeneration *in vivo*. *Brain*. (2018) 141:1753–69. doi: 10.1093/brain/awy077
28. Nielsen SR, Quaranta V, Linford A, Emeagi P, Rainer C, Santos A, et al. Macrophage-secreted granulin supports pancreatic cancer metastasis by inducing liver fibrosis. *Nat Cell Biol*. (2016) 18:549–60. doi: 10.1038/ncb3340
29. Nguyen AD, Nguyen TA, Singh RK, Eberlé D, Zhang J, Abate JP, et al. Progranulin in the hematopoietic compartment protects mice from atherosclerosis. *Atherosclerosis*. (2018) 277:145–54. doi: 10.1016/j.atherosclerosis.2018.08.042
30. Carneiro FRG, Lepelley A, Seeley JJ, Hayden MS, Ghosh S. An essential role for ECSIT in mitochondrial complex I assembly and mitophagy in macrophages. *Cell Rep*. (2018) 22:2654–66. doi: 10.1016/j.celrep.2018.02.051
31. Zhou J, Yang R, Zhang Z, Liu Q, Zhang Y, Wang Q, et al. Mitochondrial protein PINK1 positively regulates RLR signaling. *Front Immunol*. (2019) 10:1069. doi: 10.3389/fimmu.2019.01069
32. Xu Y, Shen J, Ran Z. Emerging views of mitophagy in immunity and autoimmune diseases. *Autophagy*. (2019) 15:1–15. doi: 10.1080/15548627.2019.1603547
33. Zhong Z, Umemura A, Sanchez-Lopez E, Liang S, Shalapour S, Wong J, et al. NF-κB restricts inflammasome activation via elimination of damaged mitochondria. *Cell*. (2016) 164:896–910. doi: 10.1016/j.cell.2015.12.057
34. Sliter DA, Martinez J, Hao L, Chen X, Sun N, Fischer TD, et al. Parkin and PINK1 mitigate STING-induced inflammation. *Nature*. (2018) 561:258–62. doi: 10.1038/s41586-018-0448-9
35. National Library of Medicine (US). *Genetics Home Reference*. Bethesda, MD: The Library. Available online at: https://ghr.nlm.nih.gov
36. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res*. (2016) 44:W90–7. doi: 10.1093/nar/gkw377
37. Pletscher-Frankild S, Pallejà A, Tsafou K, Binder JX, Jensen LJ. DISEASES: text mining and data integration of disease–gene associations. *Methods*. (2015) 74:83–9. doi: 10.1016/j.ymeth.2014.11.020
38. Oughtred R, Stark C, Breitkreutz B-J, Rust J, Boucher L, Chang C, et al. The BioGRID interaction database: 2019 update. *Nucleic Acids Res*. (2019) 47:D529–41. doi: 10.1093/nar/gky1079
39. Kim M-S, Pinto SM, Getnet D, Nirujogi RS, Manda SS, Chaerkady R, et al. A draft map of the human proteome. *Nature*. (2014) 509:575–81. doi: 10.1038/nature13302
40. Johnston HE, Carter MJ, Larrayoz M, Clarke J, Garbis SD, Oscier D, et al. Proteomics profiling of CLL versus healthy B-cells identifies putative therapeutic targets and a subtype-independent signature of spliceosome dysregulation. *Mol Cell Proteomics*. (2018) 17:776–91. doi: 10.1074/mcp.RA117.000539
41. Worah K, Mathan TSM, Vu Manh TP, Keerthikumar S, Schreibelt G, Tel J, et al. Proteomics of human dendritic cell subsets reveals subset-specific surface markers and differential inflammasome function. *Cell Rep*. (2016) 16:2953–66. doi: 10.1016/j.celrep.2016.08.023
42. Strasser L, Dang H-H, Schwarz H, Asam C, Ferreira F, Horejs-Hoeck J, et al. Unbiased quantitative proteomics reveals a crucial role of the allergen context for the activation of human dendritic cells. *Sci Rep*. (2017) 7:16638. doi: 10.1038/s41598-017-16726-2
43. Dhanda SK, Mahajan S, Paul S, Yan Z, Kim H, Jespersen MC, et al. IEDB-AR: immune epitope database—analysis resource in 2019. *Nucleic Acids Res*. (2019) 47:W502–6. doi: 10.1093/nar/gkz452
44. UniProt Consortium. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res*. (2019) 47:D506–15. doi: 10.1093/nar/gky1049
45. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. (2003) 13:2498–504. doi: 10.1101/gr.1239303
46. Bretou M, Kumari A, Malbec O, Moreau HD, Obino D, Pierobon P, et al. Dynamics of the membrane-cytoskeleton interface in MHC class II-restricted antigen presentation. *Immunol Rev*. (2016) 272:39–51. doi: 10.1111/imr.12429
47. Yuseff M-I, Pierobon P, Reversat A, Lennon-Duménil A-M. How B cells capture, process and present antigens: a crucial role for cell polarity. *Nat Rev Immunol*. (2013) 13:475–86. doi: 10.1038/nri3469
48. Abbas AK. Antigen presentation by B lymphocytes: mechanisms and functional significance. *Semin Immunol*. (1989) 1:5–12.
49. Villadangos JA, Schnorrer P. Intrinsic and cooperative antigen-presenting functions of dendritic-cell subsets *in vivo*. *Nat Rev Immunol*. (2007) 7:543–55. doi: 10.1038/nri2103
50. Guermonprez P, Valladeau J, Zitvogel L, Théry C, Amigorena S. Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol*. (2002) 20:621–67. doi: 10.1146/annurev.immunol.20.100301.064828
51. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol*. (2003) 21:685–711. doi: 10.1146/annurev.immunol.21.120601.141040
52. Zhou D, Li P, Lin Y, Lott JM, Hislop AD, Canaday DH, Brutkiewicz RR, et al.—2a facilitates MHC class II presentation of cytoplasmic antigens. *Immunity*. (2005) 22:571–81. doi: 10.1016/j.immuni.2005.03.009
53. Mycko MP, Cwiklinska H, Szymanski J, Szymanska B, Kudla G, Kilianek L, et al. Inducible heat shock protein 70 promotes myelin autoantigen presentation by the HLA class II. *J Immunol*. (2004) 172:202–13. doi: 10.4049/jimmunol.172.1.202
54. Houlihan JL, Metzler JJ, Blum JS. HSP90alpha and HSP90beta isoforms selectively modulate MHC class II antigen presentation in B cells. *J Immunol*. (2009) 182:7451–8. doi: 10.4049/jimmunol.0804296
55. van Eden W, Jansen MAA, Ludwig I, van Kooten P, van der Zee R, Broere F. The Enigma of heat shock proteins in immune tolerance. *Front Immunol*. (2017) 8:1599. doi: 10.3389/fimmu.2017.01599
56. Cohen IR. Autoantibody repertoires, natural biomarkers, and system controllers. *Trends Immunol*. (2013) 34:620–5. doi: 10.1016/j.it.2013.05.003
57. Quintana FJ, Cohen IR. Heat shock proteins as endogenous adjuvants in sterile and septic inflammation. *J Immunol*. (2005) 175:2777–82. doi: 10.4049/jimmunol.175.5.2777
58. Kuwana T, Peterson PA, Karlsson L. Exit of major histocompatibility complex class II-invariant chain p35 complexes from the endoplasmic reticulum is modulated by phosphorylation. *Proc Natl Acad Sci USA*. (1998) 95:1056–61. doi: 10.1073/pnas.95.3.1056
59. Buschow SI, van Balkom BWM, Aalberts M, Heck AJR, Wauben M, Stoorvogel W. MHC class II-associated proteins in B-cell exosomes and potential functional implications for exosome biogenesis. *Immunol Cell Biol*. (2010) 88:851–6. doi: 10.1038/icb.2010.64
60. Umahara T, Uchihara T, Tsuchiya K, Nakamura A, Iwamoto T, Ikeda K, et al. 14-3-3 proteins and zeta isoform containing neurofibrillary tangles in patients with Alzheimer's disease. *Acta Neuropathol*. (2004) 108:279–86. doi: 10.1007/s00401-004-0885-4
61. Kawamoto Y, Akiguchi I, Nakamura S, Honjyo Y, Shibasaki H, Budka H. 14-3-3 proteins in Lewy bodies in Parkinson disease and diffuse Lewy body disease brains. *J Neuropathol Exp Neurol*. (2002) 61:245–53. doi: 10.1093/jnen/61.3.245
62. Jia B, Wu Y, Zhou Y. 14-3-3 and aggresome formation: implications in neurodegenerative diseases. *Prion*. (2014) 8:173–7. doi: 10.4161/pri.28123
63. Magilnick N, Reyes EY, Wang W-L, Vonderfecht SL, Gohda J, Inoue J, et al. *miR-146a – Traf6* regulatory axis controls autoimmunity and myelopoiesis, but is dispensable for hematopoietic stem cell homeostasis and tumor suppression. *Proc Natl Acad Sci USA*. (2017) 114:E7140–9. doi: 10.1073/pnas.1706833114
64. Brenke JK, Popowicz GM, Schorpp K, Rothenaigner I, Roesner M, Meininger I, et al. Targeting TRAF6 E3 ligase activity with a small-molecule inhibitor combats autoimmunity. *J Biol Chem*. (2018) 293:13191–203. doi: 10.1074/jbc.RA118.002649
65. Ahonen CL, Manning EM, Erickson LD, O'Connor BP, Lind EF, Pullen SS, et al. The CD40-TRAF6 axis controls affinity maturation and the generation of long-lived plasma cells. *Nat Immunol*. (2002) 3:451–6. doi: 10.1038/ni792
66. Jalukar S V., Hostager BS, Bishop GA. Characterization of the roles of TNF receptor-associated factor 6 in CD40-Mediated B lymphocyte

effector functions. *J Immunol*. (2000) 164:623–30. doi: 10.4049/jimmunol.164.2.623
67. Ben-Nun A, Kaushansky N, Kawakami N, Krishnamoorthy G, Berer K, Liblau R, et al. From classic to spontaneous and humanized models of multiple sclerosis: Impact on understanding pathogenesis and drug development. *J Autoimmun*. (2014) 54:33–50. doi: 10.1016/j.jaut.2014.06.004
68. Adorini L, Moreno J, Momburg F, Hämmerling GJ, Guéry JC, Valli A, et al. Exogenous peptides compete for the presentation of endogenous antigens to major histocompatibility complex class II-restricted T cells. *J Exp Med*. (1991) 174:945–8. doi: 10.1084/jem.174.4.945
69. Leung CSK. Endogenous antigen presentation of MHC class II epitopes through non-autophagic pathways. *Front Immunol*. (2015) 6:464. doi: 10.3389/fimmu.2015.00464
70. Coulon P-G, Richetta C, Rouers A, Blanchet FP, Urrutia A, Guerbois M, et al. HIV-infected dendritic cells present endogenous MHC class II-restricted antigens to HIV-specific CD4+ T cells. *J Immunol*. (2016) 197:517–32. doi: 10.4049/jimmunol.1600286
71. Rudensky AY, Maric M, Eastman S, Shoemaker L, DeRoos PC, Blum JS. Intracellular assembly and transport of endogenous peptide-MHC class II complexes. *Immunity*. (1994) 1:585–94. doi: 10.1016/1074-7613(94)90048-5
72. Pertea M, Shumate A, Pertea G, Varabyou A, Breitwieser FP, Chang Y-C, et al. CHESS: a new human gene catalog curated from thousands of large-scale RNA sequencing experiments reveals extensive transcriptional noise. *Genome Biol*. (2018) 19:208. doi: 10.1186/s13059-018-1590-2
73. Bartos A, Fialová L, Švarcová J. Lower serum antibodies against tau protein and heavy neurofilament in Alzheimer's Disease. *J Alzheimers Dis*. (2018) 64:751–60. doi: 10.3233/JAD-180039
74. Taguchi H, Planque S, Nishiyama Y, Szabo P, Weksler ME, Friedland RP, et al. Catalytic antibodies to amyloid beta peptide in defense against Alzheimer disease. *Autoimmun Rev*. (2008) 7:391–7. doi: 10.1016/j.autrev.2008.03.004
75. Huang Y, Xie X, Ji M, Yu X, Zhu J, Zhang L, et al. Naturally occurring autoantibodies against α-synuclein rescues memory and motor deficits and attenuates α-synuclein pathology in mouse model of Parkinson's disease. *Neurobiol Dis*. (2019) 124:202–17. doi: 10.1016/j.nbd.2018.11.024
76. Albus A, Gold M, Bach J-P, Burg-Roderfeld M, Jördens M, Kirchhein Y, et al. Extending the functional characteristics of naturally occurring autoantibodies against β-Amyloid, Prion Protein and α-Synuclein. *PLoS ONE*. (2018) 13:e0202954. doi: 10.1371/journal.pone.0202954
77. Kuhn I, Rogosch T, Schindler TI, Tackenberg B, Zemlin M, Maier RF, et al. Serum titers of autoantibodies against α-synuclein and tau in child- and adulthood. *J Neuroimmunol*. (2018) 315:33–9. doi: 10.1016/j.jneuroim.2017.12.003
78. Krestova M, Hromadkova L, Ricny J. Purification of natural antibodies against tau protein by affinity chromatography. *Methods Mol Biol*. (2017) 1643:33–44. doi: 10.1007/978-1-4939-7180-0_4
79. Lindestam Arlehamn CS, Pham J, Alcalay RN, Frazier A, Shorr E, Carpenter C, et al. Widespread Tau-specific CD4 T cell reactivity in the general population. *J Immunol*. (2019) 203:84–92. doi: 10.4049/jimmunol.1801506
80. Cohen IR, Wekerle H. Regulation of autosensitization: the immune activation and specific inhibition of self-recognizing thymus-derived lymphocytes. *J Exp Med*. (1973) 137:224–38. doi: 10.1084/jem.137.2.224
81. Cohen IR, Wekerle H. Autosensitization of lymphocytes against thymus reticulum cells. *Science*. (1972) 176:1324–5. doi: 10.1126/science.176.4041.1324
82. Cohen IR, Globerson A, Feldman M. Autosensitization *in vitro*. *J Exp Med*. (1971) 133:834–45. doi: 10.1084/jem.133.4.834
83. Coutinho A, Kazatchkine MD, Avrameas S. Natural autoantibodies. *Curr Opin Immunol*. (1995) 7:812–8. doi: 10.1016/0952-7915(95)80053-0
84. Schwartz M, Cohen IR. Autoimmunity can benefit self-maintenance. *Immunol Today*. (2000) 21:265–8. doi: 10.1016/S0167-5699(00)01633-9
85. Schwartz M, Baruch K. Breaking peripheral immune tolerance to CNS antigens in neurodegenerative diseases: boosting autoimmunity to fight-off chronic neuroinflammation. *J Autoimmun*. (2014) 54:8–14. doi: 10.1016/j.jaut.2014.08.002
86. Kipnis J, Cardon M, Strous RD, Schwartz M. Loss of autoimmune T cells correlates with brain diseases: possible implications for schizophrenia? *Trends Mol Med*. (2006) 12:107–12. doi: 10.1016/j.molmed.2006.01.003
87. Radjavi A, Smirnov I, Derecki N, Kipnis J. Dynamics of the meningeal CD4+ T-cell repertoire are defined by the cervical lymph nodes and facilitate cognitive task performance in mice. *Mol Psychiatry*. (2014) 19:531–2. doi: 10.1038/mp.2013.79
88. Derecki NC, Cardani AN, Yang CH, Quinnies KM, Crihfield A, Lynch KR, et al. Regulation of learning and memory by meningeal immunity: a key role for IL-4. *J Exp Med*. (2010) 207:1067–80. doi: 10.1084/jem.20091419
89. Brynskikh A, Warren T, Zhu J, Kipnis J. Adaptive immunity affects learning behavior in mice. *Brain Behav Immun*. (2008) 22:861–9. doi: 10.1016/j.bbi.2007.12.008
90. Kipnis J, Cohen H, Cardon M, Ziv Y, Schwartz M. T cell deficiency leads to cognitive dysfunction: Implications for therapeutic vaccination for schizophrenia and other psychiatric conditions. *Proc Natl Acad Sci USA*. (2004) 101:8180–5. doi: 10.1073/pnas.0402268101
91. Jelcic I, Al Nimer F, Wang J, Lentsch V, Planas R, Jelcic I, et al. Memory B cells activate brain-homing, autoreactive CD4+ T cells in multiple sclerosis. *Cell*. (2018) 175:85–100.e23. doi: 10.1016/j.cell.2018.08.011
92. Schmid D, Münz C. Innate and adaptive immunity through autophagy. *Immunity*. (2007) 27:11–21. doi: 10.1016/j.immuni.2007.07.004
93. Mohme M, Hotz C, Stevanović S, Binder T, Lee J-H, Okoniewski M, et al. HLA-DR15-derived self-peptides are involved in increased autologous T cell proliferation in multiple sclerosis. *Brain*. (2013) 136:1783–98. doi: 10.1093/brain/awt108
94. Kipnis J, Avidan H, Caspi RR, Schwartz M. Dual effect of CD4+CD25+ regulatory T cells in neurodegeneration: a dialogue with microglia. *Proc Natl Acad Sci USA*. (2004) 101:14663–14669. doi: 10.1073/pnas.0404842101
95. Baruch K, Rosenzweig N, Kertser A, Deczkowska A, Sharif AM, Spinrad A, et al. Breaking immune tolerance by targeting Foxp3+ regulatory T cells mitigates Alzheimer's disease pathology. *Nat Commun*. (2015) 6:7967. doi: 10.1038/ncomms8967
96. Baek H, Ye M, Kang G-H, Lee C, Lee G, Bin D, et al. Neuroprotective effects of CD4+CD25+Foxp3+ regulatory T cells in a 3xTg-AD Alzheimer's disease model. *Oncotarget*. (2016) 7:69347–57. doi: 10.18632/oncotarget.12469
97. Dansokho C, Ait Ahmed D, Aid S, Toly-Ndour C, Chaigneau T, Calle V, et al. Regulatory T cells delay disease progression in Alzheimer-like pathology. *Brain*. (2016) 139:1237–51. doi: 10.1093/brain/awv408
98. Majcher V, Goode A, James V, Layfield R. Autophagy receptor defects and ALS-FTLD. *Mol Cell Neurosci*. (2015) 66:43–52. doi: 10.1016/j.mcn.2015.01.002
99. Ciechanover A, Kwon YT. Protein quality control by molecular chaperones in neurodegeneration. *Front Neurosci*. (2017) 11:185. doi: 10.3389/fnins.2017.00185
100. Springer W, Kahle PJ. Regulation of PINK1-parkin-mediated mitophagy. *Autophagy*. (2011) 7:266–78. doi: 10.4161/auto.7.3.14348
101. Vives-Bauza C, Przedborski S. Mitophagy: the latest problem for Parkinson's disease. *Trends Mol Med*. (2011) 17:158–65. doi: 10.1016/j.molmed.2010.11.002
102. Baden P, Deleidi M. Mitochondrial antigen presentation: a vacuolar path to autoimmunity in Parkinson's Disease. *Trends Immunol*. (2016) 37:719–21. doi: 10.1016/j.it.2016.08.016
103. Roberts RF, Fon EA. Presenting mitochondrial antigens: PINK1, Parkin and MDVs steal the show. *Cell Res*. (2016) 26:1180–1. doi: 10.1038/cr.2016.104
104. Prat E, Tomaru U, Sabater L, Park DM, Granger R, Kruse N, et al. HLA-DRB5*0101 and -DRB1*1501 expression in the multiple sclerosis-associated HLA-DR15 haplotype. *J Neuroimmunol*. (2005) 167:108–19. doi: 10.1016/j.jneuroim.2005.04.027
105. Bergström TF, Josefsson A, Erlich HA, Gyllensten U. Recent origin of HLA-DRB1 alleles and implications for human evolution. *Nat Genet*. (1998) 18:237–42. doi: 10.1038/ng0398-237
106. Nataf S. Evolution, immunity and the emergence of brain superautoantigens. *F1000Research*. (2017) 6:171. doi: 10.12688/f1000research.10950.1
107. Nataf S. Autoimmunity as a driving force of cognitive evolution. *Front Neurosci*. (2017) 11:582. doi: 10.3389/fnins.2017.00582
108. James LM, Christova P, Lewis SM, Engdahl BE, Georgopoulos A, Georgopoulos AP. Protective effect of Human Leukocyte Antigen (HLA) Allele DRB1*13:02 on age-related brain gray matter volume

reduction in healthy women. *EBioMedicine*. (2018) 29:31–7. doi: 10.1016/j.ebiom.2018.02.005

109. Wang Z-X, Wan Y, Tan L, Liu J, Wang H-F, Sun F-R, et al. Genetic association of HLA gene variants with MRI brain structure in Alzheimer's Disease. *Mol Neurobiol*. (2017) 54:3195–204. doi: 10.1007/s12035-016-9889-z
110. Nataf S, Guillen M, Pays L. Common neurodegeneration-associated proteins are physiologically expressed by antigen-presenting cells and are interconnected via the inflammation/autophagy-related proteins TRAF6 and SQSTM1. *bioRxiv*. (2019). doi: 10.1101/690677

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REVIEW
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Immunological Bases of Paraneoplastic Cerebellar Degeneration and Therapeutic Implications

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Paraneoplastic cerebellar degeneration (PCD) is a rare immune-mediated disease that develops mostly in the setting of neoplasia and offers a unique prospect to explore the interplay between tumor immunity and autoimmunity. In PCD, the deleterious adaptive immune response targets self-antigens aberrantly expressed by tumor cells, mostly gynecological cancers, and physiologically expressed by the Purkinje neurons of the cerebellum. Highly specific anti-neuronal antibodies in the serum and cerebrospinal fluid represent key diagnostic biomarkers of PCD. Some anti-neuronal antibodies such as anti-Yo autoantibodies (recognizing the CDR2/CDR2L proteins) are only associated with PCD. Other anti-neuronal antibodies, such as anti-Hu, anti-Ri, and anti-Ma2, are detected in patients with PCD or other types of paraneoplastic neurological manifestations. Importantly, these autoantibodies cannot transfer disease and evidence for a pathogenic role of autoreactive T cells is accumulating. However, the precise mechanisms responsible for disruption of self-tolerance to neuronal self-antigens in the cancer setting and the pathways involved in pathogenesis within the cerebellum remain to be fully deciphered. Although the occurrence of PCD is rare, the risk for such severe complication may increase with wider use of cancer immunotherapy, notably immune checkpoint blockade. Here, we review recent literature pertaining to the pathophysiology of PCD and propose an immune scheme underlying this disabling disease. Additionally, based on observations from patients' samples and on the pre-clinical model we recently developed, we discuss potential therapeutic strategies that could blunt this cerebellum-specific autoimmune disease.

Keywords: paraneoplastic cerebellar degeneration, anti-neuronal antibodies, T cell, autoimmunity, immunotherapy, animal model

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INTRODUCTION

The central nervous system (CNS) can be the target of deleterious cellular and humoral immune responses in context of infectious, degenerative, or autoimmune diseases (1–4). Among these immune-mediated CNS disorders, autoimmune diseases are wide and heterogeneous, occurring both in paraneoplastic and non-paraneoplastic context (1). Paraneoplastic neurological disorders are characterized by acute or subacute neurological manifestations associated with autoantibodies

against antigens expressed physiologically by neural cells as well as by tumor cells, so-called "onconeuronal antigens" (5, 6). Although the autoantibodies are considered faithful diagnostic biomarkers of paraneoplastic neurological disorders, their pathogenic contribution, when the target antigens are intracellular, is uncertain (7, 8). In these cases, antigen-specific cytotoxic CD8 T cells that recognize epitopes derived from intracellular proteins in the context of MHC class I presentation are considered the main players causing the neuronal damage (9, 10).

Paraneoplastic cerebellar degeneration (PCD), one of the most common paraneoplastic neurological syndromes (11), represents a heterogeneous group that differs in clinical features, prognosis, associated tumor and associated antibody (7) (**Table 1**).

Cerebellar degeneration is the dominant presentation but neocortex, limbic system, basal ganglia, spinal cord and the peripheral nervous system can be involved (88). The clinical presentation is partially correlated to the pattern of expression of the target autoantigen in the CNS. Antibodies are mainly directed against intracellular neuronal antigens and, contrary to autoimmune encephalitis associated with antibodies targeting cell surface proteins (such as the NMDA receptor), the pathogenic immune response involves cellular immune mechanisms and irreversible neuronal death. This neuronal death leads to severe and irreversible neurological impairment. Highly specific anti-neuronal autoantibodies in the sera and/or cerebrospinal fluid (CSF) are the key diagnostic biomarkers of PCD. About 50% of PCD cases are related with anti-Yo antibodies, also known as CDR2/CDR2L (cerebellar degeneration-related antigen), making anti-Yo antibodies the predominant autoantibody associated with PCD among the 37 other anti-neural antibodies described (**Table 1**), such as anti-Hu, anti-Tr, anti-Ri, anti-Ma2, anti-P/Q-type calcium channel, or anti-CV2/CRMP5 (89–91).

The anti-CDR2/CDR2L antibody-associated PCD will be the main focus of the current article. This type of PCD develops mostly in female patients with gynecologic (ovarian and breast) carcinomas that express the Purkinje neuron-specific CDR2 protein (9) and its paralog CDR2L (14), but also in patients with other types of cancers including endometrial, digestive and lung (37, 92–95).

EXPRESSION AND ROLE OF CDR2/CDR2L PROTEINS IN PHYSIOLOGICAL CONTEXT

CDR2 and CDR2L are members of the cerebellar degeneration related (CDR) protein family. The Cdr2 gene is widely transcribed and encodes a cytoplasmic leucine zipper protein. The RNA is expressed in almost all tissues but the protein has only been found to be expressed in cerebellar Purkinje neurons, some brainstem neurons, ovarian and mammary tissue, prostate, testis and spermatogonia (96–99). These results suggest that the tissue-specific expression of CDR2 is regulated at a post-transcriptional level.

The biological function of CDR2 remains ill defined, and that of CDR2L even less known. It has been shown that CDR2 inhibits the functions of the oncogene c-Myc, a master regulator of cellular growth and cellular metabolism, through its sequestration in the cytoplasm (100). CDR2 interacts with other proteins involved in signal transduction and gene transcription such as cell cycle-related proteins, and acid-activated serine/threonine protein kinase (100–104). A similar interaction was found with NF-kB, a transcription factor involved in neuronal development and synaptic plasticity (105); and with MRGXm, a transcriptional regulator involved in cell growth and apoptosis (103). These data indicate that CDR2 may be involved in the regulation of cell cycle, at least in part through interactions with c-Myc. Since cells display an impaired proliferation capacity upon CDR2 knockdown, it can be reasoned that CDR2 is required for appropriate mitotic function (101).

Anti-Yo antibodies react against CDR2 (62 kDa protein consisting of 454 amino acids) and CDR2L (a CDR2 paralog consisting of 465 amino acids). CDR2L has a 44.7% sequence identity with CDR2 and contains three potential coiled-coil regions (14). In the cerebellum, both CDR2 and CDR2L are present in the cytoplasm and proximal dendrites of Purkinje cells (96, 100). Recent data, combining co-staining on human cerebellar sections and cultured cancer cells, protein immunoprecipitation and pre-absorption experiments, strongly suggest that under native conditions CDR2L, rather than or in addition to CDR2, is the major target of serum and CSF Yo antibodies (14, 106). However, there is currently no evidence indicating that immunity toward CDR2L actually causes Purkinje cell death.

DISRUPTION OF IMMUNE TOLERANCE AND INITIATION OF THE AUTOIMMUNE PROCESS IN PCD

Immune Tolerance and PCD

The expression of CDR2 appears tightly restricted to "immune privileged" sites whereas CDRL2 is transcribed also in the digestive tract. CDR2 has been found to be poorly immunogenic even when expressed in tumors (107, 108). During lymphocytes development in the thymus, tolerance mechanisms delete most autoreactive T cells with high affinity or redirect them to a regulatory phenotype (109, 110). Through "ectopic" expression of a number of tissue-associated self-antigens, the autoimmune regulator (AIRE) acts as a master regulator of central T-cell tolerance by preventing the development of pathogenic autoreactive T cells (111, 112). Interestingly, transcription of both *Cdr2* and *Cdr2l* has been reported in thymic medullary epithelial cells (113). Whether this expression is dependent on AIRE and whether it results in central T-cell tolerance remains unknown at this time. In this context, the observed up-regulation of *AIRE* mRNA expression in ovarian tumors associated with anti-Yo PCD as compared to other ovarian tumors is puzzling (114). Moreover, genes transcriptionally regulated by *AIRE* are enriched among those differentially expressed between anti-Yo-associated *vs.* control ovarian tumors, although the immunological consequences of this differential expression is unknown (114).

TABLE 1 | Main autoantibodies reported in paraneoplastic cerebellar degeneration.

Autoantibodies	Target, role, and localization	Main associated tumor	References
PCD with associated autoantibodies			
Anti-Purkinje cell cytoplasmic antibody-type 1 (PCA1) (anti-Yo)	CDR2 and its paralogue CDR2L, putative neuronal signal transduction proteins, in the cytoplasm of Purkinje cells	Ovarian tumor, breast cancer	(12–14)
ANNA-1 (anti-Hu/HuD)	RNA-binding protein, cytoplasmic, neuronal nuclei	SCLC, other neuroendocrine tumors	(15–17)
Anti-GAD65	Enzyme expressed intracellularly, allowing the conversion of glutamate to GABA in CNS neurons and pancreatic islet cells	Rarely paraneoplastic if not associated with other neuronal autoantibodies: SCLC, neuroendocrine, thymoma, breast cancer, non-Hodgkin lymphoma	(18–22)
Anti-Ca/RhoGTPase-activating protein 26 (anti-ARHGAP26)	Also referred to as oligophrenin-like protein, GTPase-activating protein involved in numerous pathways (in particular in endocytic pathway). In cytosol and at the membrane of Purkinje cells, stellar cells, basket cells, Golgi cells and the granular cells in cerebellum as well as in a subset of neurons in the hippocampus	One case with ovarian carcinoma, one with history of breast cancer and malignant melanoma, one with a B-cell lymphoma, one with prostate cancer, one with gastric adenocarcinoma	(23–27)
Anti-glial fibrillary acidic protein (anti-GFAP)	Major intermediate filament protein of mature astrocytes, localized in the cytoplasm.	Ovarian teratoma, adrenal carcinoma, and others	(28)
Anti-CV2/CRMP5	Cytosolic protein involved in brain ontogenesis by relaying semaphorin 3A signaling. Located predominantly in dendrites of cortical pyramidal neurons, hippocampal CA1 pyramidal cells, Purkinje cells and oligodendrocytes	SCLC, thymoma, gynecological cancer	(29–32)
Anti-metabotropic glutamate receptor 1 (anti-mGluR1)	Main glutamate metabotropic receptor at the cell surface of Purkinje cells; but also widely expressed in the CNS	Hodgkin's lymphoma, prostate adenocarcinoma	(33–36)
Anti-voltage-gated calcium channel (anti-P/Q-type VGCC +/- anti-N-type VGCC)	Membrane high-voltage threshold-activated cation channel, mediating P- and Q-type Ca2+ currents. Important role in glutamatergic neurotransmission. Expressed on Purkinje cells somata and dendrites and abundantly throughout the CNS	SCLC (60%)	(37–39)
Anti-amphiphysin	Thought to regulate exocytosis in synapses and to control the properties of the membrane associated cytoskeleton. It is a cytoplasmic synaptic vesicle-associated protein	When not associated with other neuronal autoantibodies: breast cancer and lung carcinoma	(40, 41)
Anti-dipeptidyl peptidase-like protein 6 (anti-DPPX)	Extracellular regulatory subunit of the Kv4.2 potassium channels at the cell surface of neurons	B cell neoplasm in some patients	(42–45)
Anti-contactin-associated protein 2 (anti-caspr2)	Transmembrane protein. Essential for the clustering of the VGKC subunits Kv1.1 and Kv1.2 at juxtaparanodal regions of myelinated axons and at the axon hillock. Highly expressed in the axons of the granule neurons of the cerebellum	Rarely, thymoma	(46–48)
Anti-γ-aminobutyric acid B receptor antibodies (anti-GABAbR)	Receptor of the main inhibitory neurotransmitter, localized on the neuronal membrane	Around 50% of cases: SCLC, neuroendocrine tumor	(49–51)
AGNA/Anti-SOX1	Developmental transcription factor; preferentially expressed in Bergman glial cell nuclei	Lung cancer	(52, 53)
Anti-Ma2/Ta (PNMA2)	Ma2 could play a role in mRNA biogenesis, localized to structures that resemble nuclear bodies.	Testicular germ-cell tumors.	(54–56)
Anti-Ma1 (PNMA1)	Ma1 could play a role in mRNA biogenesis, localized to structures that resemble nuclear bodies.	Variable: SCLC and non-small cell lung cancer, colon cancer, non-Hodgkin lymphoma, breast cancer	(54, 57)
ANNA-2 (Anti-Ri)	Neuron-specific RNA-binding proteins, widely express in the CNS	Breast and gynecological cancer, SCLC	(58–60)
Anti-Purkinje cell antibody 2 (anti-PCA-2)	Target antigen not known	SCLC (10 cases)	(61)
ANNA-3	Target antigen not known; localized in the neuronal nuclei	SCLC	(62)
Anti-Zic4	C2H2-type zinc finger proteins acting as a transcriptional activator during neurogenesis; in neuronal nuclei.	Hodgkin's lymphoma, SCLC	(63, 64)

(Continued)

TABLE 1 | Continued

Autoantibodies	Target, role, and localization	Main associated tumor	References
Anti-Zic2	C2H2-type zinc finger proteins acting as a transcriptional activator during neurogenesis; in neuronal nuclei.	Mostly associated with anti-Zic4	(65)
Anti-Zic1	C2H2-type zinc finger proteins acting as a transcriptional activator during neurogenesis; in neuronal nuclei.	Always associated with anti-Zlc4	(65)
Anti-Homer protein homolog 3 (anti-Homer-3)	Linking mGluR1 and Homer-3; mainly in the cytosol of Purkinje cells.	One lung cancer but only four cases described	(66, 67)
Anti-Sj/inositol 1,4,5-trisphosphate receptor (anti-Sj/ITPR1)	Mediates intracellular Ca2+ release from the ER calcium storage after activation by mGluR1; mainly located in the membrane of the ER of Purkinje cells.	Five patients described: one breast cancer and no data available for the other four patients	(68, 69)
Anti-carbonic anhydrase-related protein VIII (anti-CARP VIII)	Limit Ca2+ efflux from the ER by reducing the affinity of ITPR1 for inositol 1,4,5-trisphosphate, in Purkinje cells (intracellular).	One melanoma and one ovarian cancer	(70, 71)
Anti-Tr/delta notch-like epidermal growth factor-related receptor (anti-Tr/DNER)	Transmembrane protein involved in the Notch pathway. Highly expressed in Purkinje cell body and dendrites but also in the hippocampus and cortex.	Hodgkin's lymphoma	(72–76)
Anti-tripartite motif-containing protein 46 (anti-TRIM46)	Protein involved in axon specification and outgrowth during early brain development and in the maintenance of microtubules. Specifically localized to the proximal axon.	2 SCLC and one without tumor	(77)
Anti-tripartite motif-containing protein 9 (anti-TRIM9)	Protein expressed widely in the CNS and localizes to cytoplasmic bodies that may be involved in axon guidance.	Lung adenocarcinoma	(78)
Anti-tripartite motif-containing protein 67 (anti-TRIM67)	Expressed in the cytoplasm, exclusively in the cerebellum and retina, involved in neuritogenesis.	Lung adenocarcinoma and melanoma	(78, 79)
Anti- glucose-regulated protein 78 (anti-GRP78)	Plays a role in proliferation, apoptosis and inflammation; expressed on the endothelial cell surface.	SCLC	(80)
Anti-Plasticity-Related Gene 5	Transmembrane protein involved in neurite outgrowth and dendritic spines formation; enriched in hippocampus and cerebellum	Squamous cell lung carcinoma	(81)
Anti-neurochondrin	Leucine-rich neuronal cytoplasmic protein probably involved in signal transduction, in the nervous system	one uterine cancer	(82, 83)
Anti-septin-5	Guanosine triphosphate (GTP)-binding neural protein involved in neurotransmitter exocytosis	non paraneoplastic but only four cases described	(84)
Example of studies with PCD and without identified autoantibodies			
	Lung, breast, lymphoma, gastronintestinal, ovary cancer		(85)
	9 patients with PCD and SCLC		(65)
	one thymic carcinoma		(86)
	39 cases with lymphoma, non-SCLC and genitourinary cancers		(87)

Collectively, these findings suggest that defects in immune tolerance induction could be implicated in the pathogenesis of PCD.

Central tolerance is only partial since lymphocytes capable of recognizing autoantigens are prevalent, even in healthy individuals (115). Therefore, in order to prevent deleterious autoimmune reactions, peripheral tolerance mechanisms are necessary, among which regulatory FOXP3+ CD4 T cells play a major role. Break of immune tolerance resulting in autoimmunity usually requires a failure of one or several tolerance checkpoints. The autoimmune response against CDR2/CDR2L antigens in the context of PCD is likely multifactorial, involving high CDR2/CDR2L expression in the tumor, a genetic predisposition, and a productive, chronic immune response toward tumor cells, as detailed below.

CDR2/CDR2L Protein Expression in Tumor and T Cells

The tumor inflammatory microenvironment has been suggested to facilitate the release of intracellular antigens resulting in abnormal exposure of self-antigens to the immune system; this provides an explanation for the numerous autoantibodies produced against intracellular antigens in cancer patients (116). However, PCD is rare even though all ovarian cancer subtypes, regardless of their association with anti-Yo antibodies and PCD, express CDR2/CDR2L (98, 99, 117). Therefore, the mere expression of CDR2/CDR2L by tumor cells is insufficient to trigger autoimmunity against Purkinje cells. The low incidence of PCD may also be linked to the lymphocyte expression of CDR2 (118).

Recently, it has been shown that ovarian tumors associated with PCD and anti-Yo antibodies differ from other ovarian

tumors. Indeed, Small and colleagues showed that tumor cells from all 25 PCD patients with anti-Yo antibodies exhibited (likely somatic) mutations and/or gains in *CDR2* and/or *CDR2L* genes, leading to higher protein expression and/or expression of proteins with missense mutations. This high rate of genetic alterations is characteristic of tumors from patients with PCD and anti-Yo antibodies, as they have not been reported in 841 other ovarian carcinomas (119). Moreover, this study demonstrates massive infiltration of PCD tumors with anti-Yo antibodies by activated immune effector cells. This suggests that genetic alterations in tumor cells trigger immune tolerance breakdown and initiation of the autoimmune disease. Comforting this hypothesis, ovarian tumors associated with PCD and anti-Yo antibodies are characterized by a higher and more frequent immune cell infiltration, including CD8 T cells, B cells, plasma cells and mature Lamp+ dendritic cells (DC), known to be associated with more efficient T cell antitumor response (120). The characterization of such DC and whether they contribute to onconeuronal antigen presentation remain to be determined.

Genetic Basis of PCD

The infrequency of anti-Yo antibody-associated PCD among patients with gynecological cancers could also reflect predisposing factors such as a genetic susceptibility. Hillary and colleagues conducted high resolution HLA class I and class II genotyping in 40 patients with PCD vs. ethnically matched controls (11). They provided evidence for association of the DRB1*13:01∼DQA1*01:03∼DQB1*06:03 haplotype with ovarian cancer-associated, but not breast cancer-associated, PCD (present in 9 of 29 cases). As HLA class II molecules present antigenic peptides to CD4 T cells, the data suggest that this T cell subset could be a major player in the onset of PCD. Significant findings were also observed with several HLA class I alleles, especially within the HLA-C locus (C*03:04, C*04:01, and C*07:01). These data indirectly suggest the involvement of CD8 T cells or NK cells in PCD pathogenesis. Other genes, in particular immune-related genes, may be at play. A genome-wide association study would be greatly beneficial in identifying these genes which may play varied roles in PCD pathogenesis; albeit, challenging, given the low prevalence of PCD.

BLOOD-BRAIN BARRIER TRANSMIGRATION INTO THE CEREBELLUM

Circulating immune cells have to cross the blood-brain barrier (BBB) to get into the CNS, involving distinct trafficking molecules at the surface of the BBB endothelial cells and on immune cells for the sequential transmigration steps: tethering, rolling, capture, adhesion and diapedesis (121). Several surface molecules expressed by T cells, such as P-selectin glycoprotein ligand-1 (PSGL1), activated leucocyte cell adhesion molecule (CD6) and integrins, contribute to these steps. PSGL1 bind to P/E-selectin on endothelial cells and mediates the initial rolling and tethering of CD4 and CD8 T cells (122). Furthermore, the α4β1 integrin interacts with vascular cell adhesion protein 1 (VCAM 1) to form strong adhesion between T cells and the endothelium (123, 124). Under inflammatory conditions, the BBB-endothelial cells up-regulate the expression of adhesion molecules (selectins and cell adhesion molecules of the immunoglobulin superfamily) (121). In experimental autoimmune encephalomyelitis (EAE), a classical animal model of multiple sclerosis, BBB-endothelial cells express CCL2, CCL19, and CCL21, which mediate firm arrest of CCR2+ monocytes and DC as well as CCR7+ CD4 T cells (125). Stimulating chemokine receptors also results in a conformational change of the cell surface integrin molecules providing increased affinity for their ligands (126).

Although cumulative evidence highlights the key role of CD8 T cells in several inflammatory CNS disorders such as PCD, the molecular cues responsible for trafficking of CD8 T cells into the CNS are less known. Interaction between PSGL1 and P-selectin contributes to the recruitment of CD8 T cells from multiple sclerosis patients to brain vessels (127). However, CD8 T-cell transmigration is not affected by blocking interactions between αLβ2/ICAM-1, PECAM-1/PECAM-1, or CCL2/CCR2 (128). Using a murine model of CNS autoimmune neuroinflammation, we showed that the migration of cytotoxic CD8 T cells to the CNS relies on the α4β1-integrin and that VCAM-1 and JAM-B expressed by BBB endothelial cells are likely implicated in this process (129). Targeting this pathway of T-cell trafficking to the CNS may hold promise in neurological diseases other than multiple sclerosis. Indeed, Natalizumab, a humanized mAb against α4 integrin, was tested in a patient with immune checkpoint inhibitor-induced encephalitis resulting in neurological improvement after 2 months of treatment (130) and in a few patients with Susac syndrome (131). It is yet to be determined if the pathogenic immune cells require α4 integrin expression to penetrate into the cerebellum during PCD.

Regional peculiarities may exist for T and B cell migration within the CNS. For example, in 2-day-old piglets the cerebellum was more permeable than the cortical regions to bilirubin (132). Moreover, it has been suggested that the expression of P-glycoprotein, a transporter essential in preventing the BBB penetration of substrates (133), is lower in the BBB of the cerebellum than in the cortex (134). It could therefore be proposed that structural or molecular peculiarities of the cerebellum may contribute to local transmigration of T cells during PCD.

IMMUNE MECHANISMS OF PCD

PCD is characterized by the selective and extensive loss of cerebellar Purkinje neurons associated with local inflammatory infiltrates reported in several studies (7, 135, 136). Depopulation of Purkinje cell axons and secondary demyelination is also prevalent. In some patients, immune infiltrates and microglial activation extend beyond the cerebellum. In the cerebellum of PCD patients, the inflammatory infiltrates are composed of CD8 T cells, macrophages, and activated microglia that

can form nodules (137–139). CD4 T cells and B cells are either absent or found in small numbers around blood vessels (137). No IgG deposition or complement activation is found in relation to the Purkinje cells (138, 139). In PCD CD8 T cells exhibited an activated phenotype with granzyme B- and perforin-containing cytolytic granules, which are sometimes polarized toward the targeted neurons (140, 141). The fact that Purkinje cells can up-regulate the expression of MHC class I molecules during an inflammatory process may provide the opportunity for CD8 T cells to recognize antigens presented by these neurons (140, 142, 143). We hypothesized that interferon-γ (IFNγ) is likely a part of the local inflammatory milieu as suggested by local up-regulation and nuclear translocation of phosphorylated STAT1 (143). Pre-clinical data have yet to show Purkinje cell destruction by T cells specific for PCD-associated autoantigens. Indeed, Ma1-specific Th1 CD4 T cells can induce encephalomyelitis but failed to induce neuronal degeneration (144). However, in mice, CD8 T cells specific for a model onco-neuronal antigen can kill neurons upon help from antigen-specific CD4 cells (145), with accumulative evidence indicating that cytotoxic T cells are likely final mediators of neuronal injury (146). However, in some instances, complete elimination of Purkinje cells is found in the absence of immune cell infiltration, which is reminiscent of burned out lesions (12, 139). Despite these data strongly arguing that CD8 T cells are the final effector cells involved in Purkinje cell demise, definitive proof is lacking.

A possible direct role of autoantibodies directed against intracellular target has been evoked in early studies (5, 147). However, passive transfer of anti-Yo or anti-Hu antibodies, including through the intracerebro-ventricular route, failed to transfer disease in animal models (5, 148–151). More recently, a direct neurotoxic role of autoantibodies was documented on cerebellar organotypic slice cultures upon incubation with anti-CDR2/anti-CDR2L or anti-Hu antibodies (13, 100, 152–154). Collectively, the studies indicate that, under these experimental conditions, uptake of anti-neuronal antibodies by neurons is possible and may result in neuronal death. From a mechanistic standpoint, both human and rabbit anti-CDR2/CDR2L antibodies applied on cerebellar organotypic slice culture were rapidly internalized by Purkinje cells and led to increased expression levels of voltage-gated calcium channel Cav2.1, protein kinase C gamma and calcium-dependent protease, calpain-2; this resulted in the decrease of arborizations of Purkinje cells (13). It was therefore suggested that this autoantibody internalization causes deregulation of cell calcium homeostasis. This, in turn, leads to neuronal dysfunction, ultimately resulting in destruction of diseased Purkinje neurons (13). Another team highlighted also on rat cerebellar slice cultures that application of anti-Yo positive IgG resulted in marked Purkinje cell death (155). This effect was reversed after adsorption of the anti-Yo antibodies with their 62kDa target antigen (153). As neuronal death preceded mononuclear cell infiltration, the autoantibodies appeared to have a direct pathogenic role. These data raise the question of whether there is anti-Yo antibody penetration across the blood-brain-barrier, or from CSF to tissue, and then inside Purkinje cells in patients with PCD.

PCD AS A SIDE EFFECT OF CANCER IMMUNOTHERAPY

As already underlined the occurrence of PCD is infrequent (85), with about 10 cases/year in France (119). The risk for paraneoplastic disease appears to increase with application of immunotherapies for cancer, most notably with use of immune checkpoint blockers (156, 157). Increasing numbers of cases of autoimmune encephalomyelitis developing within days after treatment with anti-PD1 mAb (either as a monotherapy or in combination with anti-CTLA-4) have been recently reported in patients harboring melanoma or other types of cancers (130, 158, 159), possibly identifying paraneoplastic neurological disorders as a side effect of immune checkpoint inhibitors. Moreover, we recently evaluated, experimentally, the possibility to induce PCD after CTLA-4 blockade in a mouse model in which a neo-self-antigen was expressed in both Purkinje cells and implanted breast tumor cells (146). In this context, an enhanced tumor control was obtained at the expense of autoimmune PCD. We showed that the immune checkpoint therapy in this mouse model of PCD elicits T cell migration into the cerebellum and subsequent killing of Purkinje cells (146). Therefore, by blocking an essential inhibitory immunological signal in the mouse model, it is possible to elicit PCD. Interestingly, our recent results indicate that while 84% of anti-CTLA-4-treated mice develop PCD, a much lower proportion of mice developed PCD upon anti-PD1 mAb therapy (unpublished). Recently, it was demonstrated that both anti-PD1 and anti-CTLA-4 antibodies target a subset of tumor-infiltrating T cell populations, resulting in the expansion of exhausted-like CD8 T cells (160). Remarkably, anti-CTLA-4 mAb, but not anti-PD1 mAb, modulated the CD4 effector compartment, specifically inducing the expansion of Th1-like CD4 effector cells (160). These CD4 effector T cells elicited by anti-CTLA-4 mAb improved anti-tumor responses by enhancing CD8 T cell infiltration, and cytolytic CD8 activity, demonstrating that PD-1 and CTLA-4 attenuate T cell activation though distinct molecular and cellular mechanisms.

Building on these observations, it is tempting to hypothesize that patients developing PCD carry polymorphisms, or that tumors harbor alterations, in genes related to the immune regulation pathway. A detailed next-generation sequencing analysis of ovarian or breast tumors associated with PCD-related to anti-Yo antibodies and in circulating T cells could explore whether there are alterations in immune regulation, either locally or systemically, in patients with PCD. In that respect, the transcriptomic profile of 12 ovarian cancers from anti-Yo PCD was compared with public data of 733 control ovarian tumor transcriptomes from The Cancer Genome Atlas database. A total of 5,634 genes were differentially expressed between anti-Yo PCD ovarian tumors and control ovarian tumors; among these genes, two members of the CD28 family—*CTLA4* and *ICOS*—were significantly down-regulated, implying that suppressive

functions of T cells could be altered within the anti-Yo PCD ovarian tumor microenvironment (114).

OVERALL IMMUNOLOGICAL SCENARIO FOR THE INITIATION AND DEVELOPMENT OF PCD (FIGURE 1)

Key questions regarding the site of priming and the antigenic specificity of the pathogenic cerebellum-infiltrating CD8 T cells remain to be answered. Regarding the site of priming, pathological studies indicate the presence of moderate to profound immune infiltrates in the primary tumor or metastases of PCD-associated tumors (12, 119, 135, 140). Importantly, these infiltrates were composed of CD8 T cells with cytotoxic potential, B cells, plasmabasts, and DC-LAMP+ dendritic cells (119). Intriguingly, those tumor infiltrates could sometimes form tertiary lymphoid structures, a feature recently associated with better prognosis and reponse to immune checkpoint blockade (161, 162). The identification of a high expression of CDR2 and CDR2L within anti-Yo PCD-associated tumors as well as a very high rate of *CDR2* and *CDR2L* mutations within the tumor strengthen the hypothesis of local adaptive immune activation against tumor antigens, mutated or not. A similar scenario has been described for patients with paraneoplasic scleroderma, in whom genetic alterations of the *POLR3A* locus and resulting T and B cell responses against the *POLR3A* gene product were detected in 75% of tumors but were absent from control tumors (163). In that regard, the identification of CDR2 antigen-specific CD8 T cells in the blood and CSF of PCD patients favors

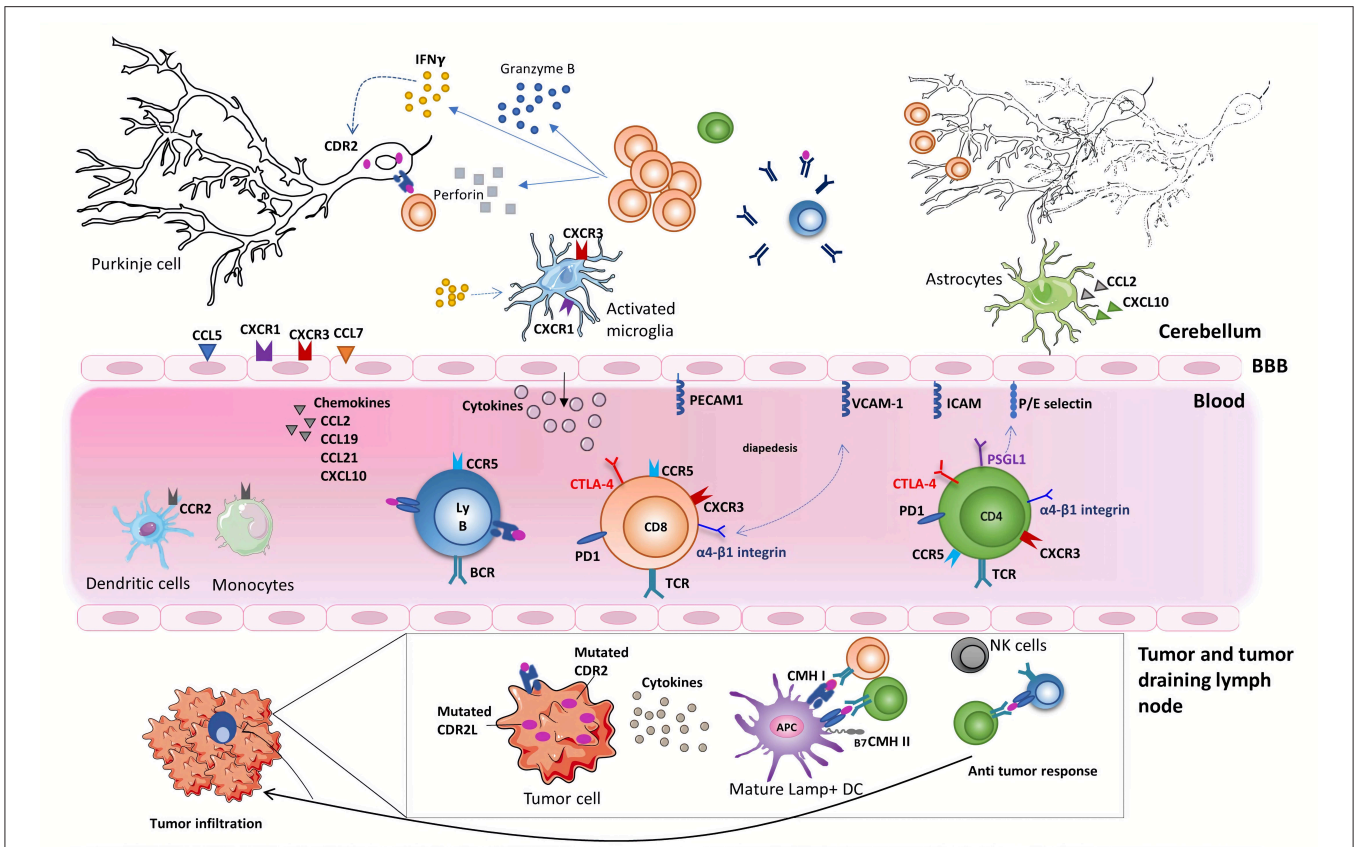

FIGURE 1 | Onconeural antigens such as CDR2 and CDR2L, mutated or not, can be the target of an anti-tumor immune response. Moderate to profound immune infiltrates are found in the primary tumor or metastases of anti-Yo PCD-associated tumors. These infiltrates sometimes form tertiary lymphoid structures. Priming of onconeural antigen-specific CD4 T cells may occur in the tumor-draining lymph nodes or the tumor itself following presentation by local DCs. Differentiation and recruitment of tumor antigen-specific cytotoxic CD8 T-cell likely contribute to the partial tumor control described in patients with PCD. B cells and plasma cells are also found in the tumor. The onconeural antigen-specific T cells reach the CNS after crossing the blood-brain barrier, through a multistep process involving selectins, integrins, and chemokine receptors. The preferential infiltration of the cerebellum in PCD patients could relate to presentation of the onconeural antigens by local APCs and/or to regional peculiarities. In the cerebellum of PCD patients, the inflammatory infiltrates are mostly composed of CD8 T cells, macrophages, and activated microglia. Purkinje neurons are naturally expressing onconeural antigen, such as CDR2 and CDR2L, and up-regulate their expression of MHC class I molecules during an inflammatory process providing the opportunity for CD8 T cells to recognize antigens presented by these neurons. Local CD8 T cells exhibit an activated phenotype with granzyme B- and perforin-containing cytolytic granules. They also secrete locally IFNγ, which has a number of disease-enhancing properties and, thus, is likely a key part of PCD pathogenesis. In particular, IFNγ increases the expression of MHC class I molecules on Purkinje neurons enhances VCAM1 expression on brain endothelial cells and elicits secretion of CXCL10, a ligand of CXCR3 expressed on Th1 cells and activated CD8 T cells. Although antibodies against intracellular onconeural antigens do not appear pathogenic *in vivo*, they could promote antigen presentation to pathogenic T cells.

the hypothesis that these T cells arise as a consequence of anti-tumor immunity (9, 164). However, the TCR repertoire and antigenic specificity of cerebellum-infiltrating CD8 T cells in PCD are still elusive. Leveraging the latest molecular tools to address these questions appears to be the next logical step (4, 165).

Several studies in PCD have focused on CD8 T cells, with much less emphasis given to CD4 T cells. In other context CD4 T cells orchestrate functional immune responses by coordinating immune activity (166). CD4 T cells optimize during the priming the cytotoxic response both in quality and scale, by increasing the cytotoxic CD8 T cell motility and migratory capacities (166). The expansion of Th1-like CD4 T cells following blockage of CTLA-4 improves anti-tumor responses by enhancing CD8 infiltration, cytotoxic CD8 T cells activity, and T cell memory formations (160). It is tempting to speculate that in PCD antigen-specific Th1-type CD4 T cells are responsible for initiating the disease, as documented in a rat model (144), and as a consequence, these CD4 T cells are able to recruit and enhance the cytotoxic CD8 T-cell responses.

We have shown in animal models as well as in cerebellar samples from PCD patients that IFNγ signaling occurs locally in Purkinje neurons and surrounding cell types (143). Moreover, CXCL10, an IFNγ-induced chemokine, is present at high level in the CSF of patients with PCD (167). We, therefore, hypothesize that IFNγ stimulates a disease-promoting cascade of events in the cerebellum and could represent a promising therapeutic target in PCD. Indeed, autoantigen-reactive CD8 T cells (and maybe other immune cell types) produce IFNγ locally, which has a number of disease-enhancing properties. IFNγ increases the expression of MHC class I molecules on neurons, including Purkinje cells, which can therefore present self-peptides to the cytotoxic CD8 T cells, promoting their own destruction (143, 168, 169). In addition, IFNγ promotes local immune cell recruitment by enhancing VCAM1 expression on brain endothelial cells and by eliciting secretion of the chemokine CXCL10, a ligand of CXCR3 expressed on Th1 cells and activated CD8 T cells. Importantly, the levels of CXCL10, but not those of CCL2, are elevated in the CSF of PCD patients indirectly suggesting that the CXCL10/CXCR3 axis may contribute to the trafficking of T cells into the cerebellum (167).

The role played by activated microglia and microglia nodules in the disease process is currently unknown. In Rasmussen encephalitis, microglial nodules have been associated with neuronal phagocytosis, following CD8 T cell-mediated brain neuron destruction (170). In human PCD, similar to Rasmussen encephalitis, IFNγ-mediated signaling (STAT1 phosphorylation) occurs in microglial cells, which can further amplify cytokine/chemokine release (143, 170). Further investigation on the role of microglia as an amplifier of inflammation and an executor of neuronal death is needed in PCD.

As already discussed, antibodies against neuronal autoantigens such as anti-Yo antibodies are extremely useful diagnostic biomarkers. Their direct pathogenic potential documented in *in vitro* models is still unproven *in vivo* (5, 13, 149, 153, 171). However, autoreactive B cells could participate in the development of the disease. They can uptake (in an antigen-specific manner due to their surface immunoglobulin), process and present autoantigens to pathogenic T cells. Their scarcity in the cerebellum would, however, rather favor an alternative scenario. For instance, autoantibodies entering the CNS can be up-taken together with their target antigen by resident antigen-presenting phagocytes, a phenomenon that enhances the activation of the incoming effector T cells (172). Therefore, the autoantibodies could cooperate with the T cells and support local autoimmune neuroinflammation.

POTENTIAL THERAPEUTIC IMPLICATIONS

To date, treatment of PCD is empirical and usually relies on 2 pillars: treatment of the underlying cancer and general immunosuppressive drugs. In the absence of large clinical trials, most of the therapeutics conclusions come from observational clinical studies and case reports. Concerning PCD associated with anti-Yo antibodies, corticoids seem ineffective, whereas plasma exchange and rituximab may have provided some benefit (173–176). The efficacy of intravenous immunoglobulins is controversial: suggested in a small proportion of patients for some studies (177, 178) but not confirmed in a larger study (89). Since there is currently no evidence arguing for a direct role of anti-Yo antibodies in Purkinje cell death, we do not address here potential therapeutic strategies aiming at reducing antibodies levels.

Due to the intracellular localization of the CDR2/CDR2L and Hu antigens, as well as the identification of CD8 T cells in close proximity to neuronal cells, cytotoxic T cells are considered to be the final effectors responsible for neuronal loss in PCD (9, 140, 146, 164, 179). These T cells likely contribute to the tumor control outside of the CNS. Therefore, the conundrum is how to selectively target the CNS-targeting immune cells while preserving (as much as possible) the tumor-controlling counterparts. Uncoupling these two simultaneous immune responses is, theoretically, possible provided that the molecular bases of immune cell migration or effector mechanisms at the two sites differ. It is paramount that the treatment of PCD should be started as early as possible since Purkinje cells are post-mitotic cells that do not renew.

The α4β1 integrin is important for both CD4 and CD8 T cell migration to the CNS and a monoclonal antibody targeting the α4 subunit provides important benefits for the treatment of persons with multiple sclerosis. Therefore, one approach could be to initiate anti-α4 integrin therapy early on in the disease process in order to preserve as many Purkinje neurons as possible. One conceptual obstacle is that blood-borne T cells may not be needed to fuel ongoing tissue destruction by tissue-resident T cells. This may explain why, in our mouse model of PCD, blocking α4 integrin early in the disease process did not yield significant benefit (143). Along the same lines, blocking CXCR3 with either monoclonal antibodies or pharmacological compounds is tempting, given the evidence of a role for the CXCL10/CXCR3 axis in PCD. However, to our knowledge, this approach has not yet reached the clinical setting.

We argued earlier that IFNγ secretion by CD8 T cells could sustain a feed forward loop by rendering Purkinje cells vulnerable to direct killing by autoantigen-specific CD8 T cells. If IFNγ plays a non-redundant role in the progression of PCD, this molecule could be targeted therapeutically. Our recent pre-clinical data showing that blocking IFNγ strongly reduces PCD development without eliciting tumor growth rebound are encouraging (143). Moreover, administration of anti-IFNγ antibodies has been tested recently in clinical trials. For instance, Emapalumab, a fully human monoclonal neutralizing anti-IFNγ antibody has been approved for the treatment of primary hemophagocytic lymphohistiocytosis, a syndrome of excessive immune activation and progressive immune-mediated organ damage due to genetic defects in cell-mediated cytotoxicity (180). Taken together, the accumulating evidence from human samples and the mouse model as well as the previous development of approved anti-IFNγ antibody in humans should facilitate the testing of this strategy in patients with PCD.

CONCLUSION

The precise mechanisms and pathways involved in the pathogenesis of PCD need to be explored in greater depth. This can be done with the use of bodily fluids (blood and CSF) and tissue from PCD patients and further validated in reductionist mouse models. Key questions remain regarding the antigenic specificity, phenotype, migration of T and B cells infiltrating the tumor and the cerebellar tissue. The molecular dissection of the steps involved in pathogenesis is a pre-requisite for rational development of new therapeutic strategies. In depth investigation of the immune changes in patients suffering from paraneoplastic neurological disorders in the frame of immune checkpoint blockade should provide clues in that respect.

AUTHOR CONTRIBUTIONS

LY and CB drafted the work. RL designed the concept and revised the manuscript critically. All authors made substantial contributions to the conception or design of the work and contributed to manuscript revision, read, and approved the submitted version.

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REFERENCES

1. Waisman A, Liblau RS, Becher B. Innate and adaptive immune responses in the CNS. *Lancet Neurol.* (2015) 14:945–55. doi: 10.1016/S1474-4422(15)00141-6
2. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, et al. T cells of Parkinson's disease patients recognize α-synuclein peptides. *Nature.* (2017) 546:656–61. doi: 10.1038/nature22815
3. Latorre D, Kallweit U, Armentani E, Foglierini M, Mele F, Cassotta A, et al. T cells in patients with narcolepsy target self-antigens of hypocretin neurons. *Nature.* (2018) 562:63–8. doi: 10.1038/s41586-018-0540-1
4. Gate D, Saligrama N, Leventhal O, Yang AC, Unger MS, Middeldorp J, et al. Clonally expanded CD8 T cells patrol the cerebrospinal fluid in Alzheimer's disease. *Nature.* (2020) 577:399–404. doi: 10.1038/s41586-019-1895-7
5. Graus F, Illa I, Agusti M, Ribalta T, Cruz-Sanchez F, Juarez C. Effect of intraventricular injection of an anti-Purkinje cell antibody (anti-Yo) in a guinea pig model. *J Neurol Sci.* (1991) 106:82–7. doi: 10.1016/0022-510X(91)90198-G
6. Furneaux HM, Rosenblum MK, Dalmau J, Wong E, Woodruff P, Graus F, et al. Selective expression of Purkinje cell antigens in tumor tissue from patients with paraneoplastic cerebellar degeneration. *N Engl J Med.* (1990) 322:1844–51. doi: 10.1056/NEJM199006283222604
7. Dalmau J, Rosenfeld MR. Paraneoplastic syndromes of the CNS. *Lancet Neurol.* (2008) 7:327–40. doi: 10.1016/S1474-4422(08)70060-7
8. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy. *Sci Transl Med.* (2016) 8:328rv4. doi: 10.1126/scitranslmed.aad7118
9. Albert ML, Darnell JC, Bender A, Francisco LM, Bhardwaj N, Darnell RB. Tumor-specific killer cells in paraneoplastic cerebellar degeneration. *Nat Med.* (1998) 4:1321–4. doi: 10.1038/3315
10. Roberts WK, Deluca IJ, Thomas A, Fak J, Williams T, Buckley N, et al. Patients with lung cancer and paraneoplastic Hu syndrome harbor HuD-specific type 2 CD8+ T cells. *J Clin Invest.* (2009) 119:2042–51. doi: 10.1172/JCI36131
11. Hillary RP, Ollila HM, Lin L, Desestret V, Rogemond V, Picard G, et al. Complex HLA association in paraneoplastic cerebellar ataxia with anti-Yo antibodies. *J Neuroimmunol.* (2018) 315:28–32. doi: 10.1016/j.jneuroim.2017.12.012
12. Peterson K, Rosenblum MK, Kotanides H, Posner JB. Paraneoplastic cerebellar degeneration : I. A clinical analysis of 55 anti-Yo antibody-positive patients. *Neurology.* (1992) 42:1931–7. doi: 10.1212/WNL.42.10.1931
13. Schubert M, Panja D, Haugen M, Bramham CR, Vedeler CA. Paraneoplastic CDR2 and CDR2L antibodies affect Purkinje cell calcium homeostasis. *Acta Neuropathol.* (2014) 128:835–52. doi: 10.1007/s00401-014-1351-6
14. Eichler TW, Totland C, Haugen M, Qvale TH, Mazengia K, Storstein A, et al. CDR2L antibodies : a new player in paraneoplastic cerebellar degeneration. *PLoS ONE.* (2013) 8:e66002. doi: 10.1371/journal.pone.0066002
15. Lucchinetti CF, Kimmel DW, Lennon VA. Paraneoplastic and oncologic profiles of patients seropositive for type 1 antineuronal nuclear autoantibodies. *Neurology.* (1998) 50:652–7. doi: 10.1212/WNL.50.3.652
16. Graus F, Keime-Guibert F, Rene R, Benyahia B, Ribalta T, Ascaso C, et al. Anti-Hu-associated paraneoplastic encephalomyelitis : analysis of 200 patients. *Brain.* (2001) 124:1138–48. doi: 10.1093/brain/124.6.1138
17. Dalmau J, Graus F, Rosenblum M, Posner JB. Anti-Hu-associated paraneoplastic encephalomyelitis/sensory neuronopathy : a clinical study of 71 patients. *Medicine (Baltimore).* (1992) 71:59–72. doi: 10.1097/00005792-199203000-00001
18. Honnorat J, Saiz A, Giometto B, Vincent A, Brieva L, de Andres C, et al. Cerebellar ataxia with anti-glutamic acid decarboxylase antibodies: study of 14 patients. *Arch Neurol.* (2001) 58:225–30. doi: 10.1001/archneur.58.2.225
19. Jarius S, Stich O, Speck J, Rasiah C, Wildemann B, Meinck HM, et al. Qualitative and quantitative evidence of anti-glutamic acid decarboxylase-specific intrathecal antibody synthesis in patients with stiff person syndrome. *J Neuroimmunol.* (2010) 229:219–24. doi: 10.1016/j.jneuroim.2010.07.019

20. Kono S, Miyajima H, Sugimoto M, Suzuki Y, Takahashi Y, Hishida A. Stiff-person syndrome associated with cerebellar ataxia and high glutamic acid decarboxylase antibody titer. *Intern Med.* (2001) 40:968–71. doi: 10.2169/internalmedicine.40.968
21. Piccolo G, Tavazzi E, Cavallaro T, Romani A, Scelsi R, Martino G. Clinico-pathological findings in a patient with progressive cerebellar ataxia, autoimmune polyendocrine syndrome, hepatocellular carcinoma and anti-GAD autoantibodies. *J Neurol Sci.* (2010) 290:148–9. doi: 10.1016/j.jns.2009.12.006
22. Saiz A, Blanco Y, Sabater L, González F, Bataller L, Casamitjana R, et al. Spectrum of neurological syndromes associated with glutamic acid decarboxylase antibodies: diagnostic clues for this association. *Brain.* (2008) 131:2553–63. doi: 10.1093/brain/awn183
23. Jarius S, Wandinger KP, Horn S, Heuer H, Wildemann B. A new Purkinje cell antibody (anti-Ca) associated with subacute cerebellar ataxia: immunological characterization. *J Neuroinflammation.* (2010) 7:21. doi: 10.1186/1742-2094-7-21
24. Jarius S, Martínez-García P, Hernandez AL, Brase JC, Borowski K, Regula JU, et al. Two new cases of anti-Ca (anti-ARHGAP26/GRAF) autoantibody-associated cerebellar ataxia. *J Neuroinflammation.* (2013) 10:7. doi: 10.1186/1742-2094-10-7
25. Doss S, Nümann A, Ziegler A, Siebert E, Borowski K, Stöcker W, et al. Anti-Ca/anti-ARHGAP26 antibodies associated with cerebellar atrophy and cognitive decline. *J Neuroimmunol.* (2014) 267:102–4. doi: 10.1016/j.jneuroim.2013.10.010
26. Bartels F, Prüss H, Finke C. Anti-ARHGAP26 autoantibodies are associated with isolated cognitive impairment. *Front Neurol.* (2018) 9:656. doi: 10.3389/fneur.2018.00656
27. Wallwitz U, Brock S, Schunck A, Wildemann B, Jarius S, Hoffmann F. From dizziness to severe ataxia and dysarthria: new cases of anti-Ca/ARHGAP26 autoantibody-associated cerebellar ataxia suggest a broad clinical spectrum. *J Neuroimmunol.* (2017) 309:77–81. doi: 10.1016/j.jneuroim.2017.05.011
28. Shan F, Long Y, Qiu W. Autoimmune glial fibrillary acidic protein astrocytopathy: a review of the literature. *Front Immunol.* (2018) 9:2802. doi: 10.3389/fimmu.2018.02802
29. Yu Z, Kryzer TJ, Griesmann GE, Kim KK, Benarroch EE, Lennon VA. CRMP-5 neuronal autoantibody: marker of lung cancer and thymoma-related autoimmunity. *Ann Neurol.* (2001) 49:146–54. doi: 10.1002/1531-8249(20010201)49:2<146::aid-ana34>3.0.co
30. Antoine JC, Honnorat J, Vocanson C, Koenig F, Aguera M, Belin MF, et al. Posterior uveitis, paraneoplastic encephalomyelitis and auto-antibodies reacting with developmental protein of brain and retina. *J Neurol Sci.* (1993) 117:215–23. doi: 10.1016/0022-510X(93)90176-Y
31. Honnorat J, Cartalat-Carel S, Ricard D, Camdessanche JP, Carpentier AF, Rogemond V, et al. Onco-neural antibodies and tumour type determine survival and neurological symptoms in paraneoplastic neurological syndromes with Hu or CV2/CRMP5 antibodies. *J Neurol Neurosurg Psychiatry.* (2009) 80:412–6. doi: 10.1136/jnnp.2007.138016
32. Honnorat J, Antoine JC, Derrington E, Aguera M, Belin MF. Antibodies to a subpopulation of glial cells and a 66 kDa developmental protein in patients with paraneoplastic neurological syndromes. *J Neurol Neurosurg Psychiatry.* (1996) 61:270–8. doi: 10.1136/jnnp.61.3.270
33. Lopez-Chiriboga AS, Komorowski L, Kümpfel T, Probst C, Hinson SR, Pittock SJ, et al. Metabotropic glutamate receptor type 1 autoimmunity : clinical features and treatment outcomes. *Neurology.* (2016) 86:1009–13. doi: 10.1212/WNL.0000000000002476
34. Marignier R, Chenevier F, Rogemond V, Sillevis Smitt P, Renoux C, Cavillon G, et al. Metabotropic glutamate receptor type 1 autoantibody–associated cerebellitis. *Arch Neurol.* (2010) 67:627–30. doi: 10.1001/archneurol.2010.51
35. Sillevis-Smitt P, Kinoshita M, De Leeuw B, Moll W, Coesmans M, Jaarsma D, et al. Paraneoplastic cerebellar ataxia due to autoantibodies against glutamate receptor. *N Engl J Med.* (2000) 342:21–7. doi: 10.1056/NEJM200001063420104
36. Iorio R, Damato V, Mirabella M, Vita MG, Hulsenboom E, Plantone D, et al. Cerebellar degeneration associated with mGluR1 autoantibodies as a paraneoplastic manifestation of prostate adenocarcinoma. *J Neuroimmunol.* (2013) 263:155–8. doi: 10.1016/j.jneuroim.2013.07.015
37. Mason WP, Graus F, Lang B, Honnorat J, Delattre JY, Valldeoriola F, et al. Small-cell lung cancer, paraneoplastic cerebellar degeneration and the Lambert-Eaton myasthenic syndrome. *Brain.* (1997) 120:1279–300. doi: 10.1093/brain/120.8.1279
38. Graus F, Lang B, Pozo-Rosich P, Saiz A, Casamitjana R, Vincent A. P / Q type calcium- channel antibodies in paraneoplastic cerebellar degeneration with lung cancer. *Neurology.* (2002) 59:764–6. doi: 10.1212/WNL.59.5.764
39. Bürk K, Wick M, Roth G, Decker P, Voltz R. Antineuronal antibodies in sporadic late-onset cerebellar ataxia. *J Neurol.* (2010) 257:59–62. doi: 10.1007/s00415-009-5262-8
40. Pittock SJ, Lucchinetti CF, Parisi JE, Benarroch EE, Mokri B, Stephan CL, et al. Amphiphysin autoimmunity: paraneoplastic accompaniments. *Ann Neurol.* (2005) 58:96–107. doi: 10.1002/ana.20529
41. Moon J, Lee S-T, Shin J-W, Byun J-I, Lim J-A, Shin Y-W, et al. Non-stiff anti-amphiphysin syndrome: clinical manifestations and outcome after immunotherapy. *J Neuroimmunol.* (2014) 274:209–14. doi: 10.1016/j.jneuroim.2014.07.011
42. Balint B, Jarius S, Nagel S, Haberkorn U, Probst C, Blöcker IM, et al. Progressive encephalomyelitis with rigidity and myoclonus: a new variant with DPPX antibodies. *Neurology.* (2014) 82:1521–8. doi: 10.1212/WNL.0000000000000372
43. Boronat A, Gelfand JM, Gresa-Arribas N, Jeong HY, Walsh M, Roberts K, et al. Encephalitis and antibodies to dipeptidyl-peptidase-like protein-6, a subunit of Kv4.2 potassium channels. *Ann Neurol.* (2013) 73:120–8. doi: 10.1002/ana.23756
44. Tobin WO, Lennon VA, Komorowski L, Probst C, Clardy SL, Aksamit AJ, et al. DPPX potassium channel antibody: Frequency, clinical accompaniments, and outcomes in 20 patients. *Neurology.* (2014) 83:1797–803. doi: 10.1212/WNL.0000000000000991
45. Stoeck K, Carstens PO, Jarius S, Raddatz D, Stöcker W, Wildemann B, et al. Prednisolone and azathioprine are effective in DPPX antibody-positive autoimmune encephalitis. *Neurol Neuroimmunol NeuroInflamm.* (2015) 2:e86. doi: 10.1212/NXI.0000000000000086
46. Becker EBE, Zuliani L, Pettingill R, Lang B, Waters P, Dulneva A, et al. Contactin-associated protein-2 antibodies in non-paraneoplastic cerebellar ataxia. *J Neurol Neurosurg Psychiatry.* (2012) 83:437–40. doi: 10.1136/jnnp-2011-301506
47. Balint B, Regula JU, Jarius S, Wildemann B. Caspr2 antibodies in limbic encephalitis with cerebellar ataxia, dyskinesias and myoclonus. *J Neurol Sci.* (2013) 327:73–4. doi: 10.1016/j.jns.2013.01.040
48. Kannoth S, Nambiar V, Gopinath S, Anandakuttan A, Mathai A, Rajan PK. Expanding spectrum of contactin-associated protein 2 (CASPR2) autoimmunity—syndrome of parkinsonism and ataxia. *Neurol Sci.* (2018) 39:455–60. doi: 10.1007/s10072-017-3222-0
49. Boronat A, Sabater L, Saiz A, Dalmau J, Graus F. GABA(B) receptor antibodies in limbic encephalitis and anti-GAD-associated neurologic disorders. *Neurology.* (2011) 76:795–800. doi: 10.1212/WNL.0b013e31820e7b8d
50. Lancaster E, Lai M, Peng X, Hughes E, Constantinescu R, Raizer J, et al. Antibodies to the GABA(B) receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. *Lancet Neurol.* (2010) 9:67–76. doi: 10.1016/S1474-4422(09)70324-2
51. Jarius S, Steinmeyer F, Knobel A, Streitberger K, Hotter B, Horn S, et al. GABA B receptor antibodies in paraneoplastic cerebellar ataxia. *J Neuroimmunol.* (2013) 256:94–6. doi: 10.1016/j.jneuroim.2012.12.006
52. Graus F, Vincent A, Pozo-Rosich P, Sabater L, Saiz A, Lang B, et al. Anti-glial nuclear antibody: marker of lung cancer-related paraneoplastic neurological syndromes. *J Neuroimmunol.* (2005) 165:166–71. doi: 10.1016/j.jneuroim.2005.03.020
53. Titulaer MJ, Klooster R, Potman M, Sabater L, Graus F, Hegeman IM, et al. SOX antibodies in small-cell lung cancer and Lambert-Eaton myasthenic syndrome: frequency and relation with survival. *J Clin Oncol.* (2009) 27:4260–7. doi: 10.1200/JCO.2008.20.6169
54. Hoffmann LA, Jarius S, Pellkofer HL, Schueller M, Krumbholz M, Koenig F, et al. Anti-Ma and anti-Ta associated paraneoplastic neurological syndromes: 22 newly diagnosed patients and review of previous cases. *J Neurol Neurosurg Psychiatry.* (2008) 79:767–73. doi: 10.1136/jnnp.2007.118588

55. Dalmau J, Graus F, Villarejo A, Posner JB, Blumenthal D, Thiessen B, et al. Clinical analysis of anti-Ma2-associated encephalitis. *Brain.* (2004) 127:1831–44. doi: 10.1093/brain/awh203
56. Voltz R, Gultekin SH, Rosenfeld MR, Gerstner E, Eichen J, Posner JB, et al. A serologic marker of paraneoplastic limbic and brain-stem encephalitis in patients with testicular cancer. *N Engl J Med.* (1999) 340:1788–95. doi: 10.1056/NEJM199906103402303
57. Dalmau J, Gultekin SH, Voltz R, Hoard R, DesChamps T, Balmaceda C, et al. Ma1, a novel neuron- and testis-specific protein, is recognized by the serum of patients with paraneoplastic neurological disorders. *Brain.* (1999) 122:27–39. doi: 10.1093/brain/122.1.27
58. Pittock SJ, Lucchinetti CF, Lennon VA. Anti-neuronal nuclear autoantibody type 2: paraneoplastic accompaniments. *Ann Neurol.* (2003) 53:580–7. doi: 10.1002/ana.10518
59. Budde-Steffen C, Anderson NE, Rosenblum MK, Graus F, Ford D, Synek BJL, et al. An antineuronal autoantibody in paraneoplastic opsoclonus. *Ann Neurol.* (1988) 23:528–31. doi: 10.1002/ana.410230518
60. O'Leary CG, Battley JE, O'Reilly S. Anti-Ri antibody associated small cell lung carcinoma. *Ir J Med Sci.* (2016) 186:265–7. doi: 10.1007/s11845-016-1402-1
61. Vernino S, Lennon VA. New Purkinje cell antibody (PCA-2): marker of lung cancer-related neurological autoimmunity. *Ann Neurol.* (2000) 47:297–305. doi: 10.1002/1531-8249(200003)47:3<297::AID-ANA4>3.0.CO;2-4
62. Chan KH, Vernino S, Lennon VA. ANNA-3 anti-neuronal nuclear antibody: marker of lung cancer-related autoimmunity. *Ann Neurol.* (2001) 50:301–11. doi: 10.1002/ana.1127
63. Bataller L, Wade DF, Fuller GN, Rosenfeld MR, Dalmau J. Cerebellar degeneration and autoimmunity to zinc-finger proteins of the cerebellum. *Neurology.* (2002) 59:1985–7. doi: 10.1212/01.WNL.0000038352.01415.CE
64. Bataller L, Wade DF, Graus F, Stacey HD, Rosenfeld MR, Dalmau J. Antibodies to Zic4 in paraneoplastic neurologic disorders and small-cell lung cancer. *Neurology.* (2004) 62:778–82. doi: 10.1212/01.WNL.0000113749.77217.01
65. Sabater L, Bataller L, Suarez-Calvet M, Saiz A, Dalmau J, Graus F. ZIC antibodies in paraneoplastic cerebellar degeneration and small cell lung cancer. *J Neuroimmunol.* (2008) 201–2:163–5. doi: 10.1016/j.jneuroim.2008.01.018
66. Zuliani L, Sabater L, Saiz A, Baiges J, Giometto B, Graus F. Homer 3 autoimmunity in subacute idiopathic cerebellar ataxia. *Neurology.* (2007) 68:239–40. doi: 10.1212/01.wnl.0000251308.79366.f9
67. Höftberger R, Sabater L, Ortega A, Dalmau J, Graus F. Patient with homer-3 antibodies and cerebellitis. *JAMA Neurol.* (2013) 70:506–9. doi: 10.1001/jamaneurol.2013.1955
68. Jarius S, Scharf M, Begemann N, Stöcker W, Probst C, Serysheva II, et al. Antibodies to the inositol 1,4,5-trisphosphate receptor type 1 (ITPR1) in cerebellar ataxia. *J Neuroinflammation.* (2014) 11:206. doi: 10.1186/s12974-014-0206-3
69. Berzero G, Hacohen Y, Komorowski L, Scharf M, Dehais C, Leclercq D, et al. Paraneoplastic cerebellar degeneration associated with anti-ITPR1 antibodies. *Neurol Neuroimmunol NeuroInflammation.* (2017) 4:e326. doi: 10.1212/NXI.0000000000000326
70. Bataller L, Sabater L, Saiz A, Serra C, Claramonte B, Graus F. Carbonic anhydrase-related protein VIII: autoantigen in paraneoplastic cerebellar degeneration. *Ann Neurol.* (2004) 56:575–9. doi: 10.1002/ana.20238
71. Höftberger R, Sabater L, Velasco F, Ciordia R, Dalmau J, Graus F. Carbonic anhydrase-related protein VIII antibodies and paraneoplastic cerebellar degeneration. *Neuropathol Appl Neurobiol.* (2014) 40:650–3. doi: 10.1111/nan.12118
72. de Graaff E, Maat P, Hulsenboom E, van Den Berg R, van Den Bent M, Demmers J, et al. Identification of delta/notch-like epidermal growth factor-related receptor as the Tr antigen in paraneoplastic cerebellar degeneration. *Ann Neurol.* (2012) 71:815–24. doi: 10.1002/ana.23550
73. Probst C, Komorowski L, de Graaff E, van Coevorden-Hameete M, Rogemond V, Honnorat J, et al. Standardized test for anti-Tr/DNER in patients with paraneoplastic cerebellar degeneration. *Neurol Neuroimmunol Neuroinflamm.* (2015) 2:e68. doi: 10.1212/NXI.0000000000000068
74. Graus F, Dalmau J, Valldeoriola F, Ferrer I, Reñé R, Marin C, et al. Immunological characterization of a neuronal antibody (anti-Tr) associated with paraneoplastic cerebellar degeneration and Hodgkin's disease. *J Neuroimmunol.* (1997) 74:55–61. doi: 10.1016/S0165-5728(96)00205-6
75. Trotter JL, Hendin BA, Osterland K. Cerebellar degeneration with Hodgkin's disease. *Arch Neurol.* (1976) 33:660–1. doi: 10.1001/archneur.1976.00500090066014
76. Bernal F, Shams'ili S, Rojas I, Sanchez-Valle R, Saiz A, Dalmau J, et al. Anti-Tr antibodies as markers of paraneoplastic cerebellar degeneration and Hodgkin's disease. *Neurology.* (2003) 60:230–4. doi: 10.1212/01.WNL.0000041495.87539.98
77. van Coevorden-Hameete MH, van Beuningen SFB, Perrenoud M, Will LM, Hulsenboom E, Demonet JF, et al. Antibodies to TRIM46 are associated with paraneoplastic neurological syndromes. *Ann Clin Transl Neurol.* (2017) 4:680–6. doi: 10.1002/acn3.396
78. Do LD, Gupton SL, Tanji K, Bastien J, Brugière S, Couté Y, et al. TRIM9 and TRIM67 are new targets in paraneoplastic cerebellar degeneration. *Cerebellum.* (2019) 18:245–54. doi: 10.1007/s12311-018-0987-5
79. Larman HB, Zhao Z, Laserson U, Li MZ, Ciccia A, Gakidis MAM, et al. Autoantigen discovery with a synthetic human peptidome. *Nat Biotechnol.* (2011) 29:535–41. doi: 10.1038/nbt.1856
80. Shimizu F, Takeshita Y, Sano Y, Hamamoto Y, Shiraishi H, Sato T, et al. GRP78 antibodies damage the blood-brain barrier and relate to cerebellar degeneration in Lambert-Eaton myasthenic syndrome. *Brain.* (2019) 142:2253–64. doi: 10.1093/brain/awz168
81. van Coevorden-Hameete MH, de Graaff E, Titulaer MJ, Hulsenboom E, Sabater L, Hoogenraad CC, et al. Plasticity-related gene 5: a novel surface autoantigen in paraneoplastic cerebellar degeneration. *Neurol Neuroimmunol NeuroInflamm.* (2015) 2:e156. doi: 10.1212/NXI.0000000000000156
82. Shelly S, Kryzer TJ, Komorowski L, Miske R, Anderson MD, Flanagan EP, et al. Neurochondrin neurological autoimmunity. *Neurol Neuroimmunol Neuroinflamm.* (2019) 6:e612. doi: 10.1212/NXI.0000000000000612
83. Miske R, Gross CC, Scharf M, Golombeck KS, Hartwig M, Bhatia U, et al. Neurochondrin is a neuronal target antigen in autoimmune cerebellar degeneration. *Neurol Neuroimmunol NeuroInflamm.* (2017) 4:e307. doi: 10.1212/NXI.0000000000000307
84. Honorat JA, Sebastian Lopez-Chiriboga A, Kryzer TJ, Fryer JP, Devine M, Flores A, et al. Autoimmune septin-5 cerebellar ataxia. *Neurol Neuroimmunol NeuroInflamm.* (2018) 5:e474. doi: 10.1212/NXI.0000000000000474
85. Vogrig A, Gigli GL, Segatti S, Corazza E, Marini A, Bernardini A, et al. Epidemiology of paraneoplastic neurological syndromes: a population-based study. *J Neurol.* (2020) 267:26–35. doi: 10.1007/s00415-019-09544-1
86. Garcia Cuartero I, Rodriguez Galdeano M, Perez Pinar M, Solis Garcia del Pozo J. Thymic carcinoma associated with cerebellar degeneration. *Eur J Case Rep Intern Med.* (2018) 5:000780. doi: 10.12890/2018_000780
87. Ducray F, Demarquay G, Graus F, Decullier E, Antoine JC, Giometto B, et al. Seronegative paraneoplastic cerebellar degeneration: the PNS Euronetwork experience. *Eur J Neurol.* (2014) 21:731–5. doi: 10.1111/ene.12368
88. Joubert B, Rostásy K, Honnorat J. Immune-mediated ataxias. *Handb Clin Neurol.* (2018) 155:313–32. doi: 10.1016/B978-0-444-64189-2.00021-4
89. Shams'ili S, Grefkens J, de Leeuw B, van den Bent M, Hooijkaas H, van der Holt B, et al. Paraneoplastic cerebellar degeneration associated with antineuronal antibodies: analysis of 50 patients. *Brain.* (2003) 126:1409–18. doi: 10.1093/brain/awg133
90. Jarius S, Wildemann B. "Medusa-head ataxia": the expanding spectrum of Purkinje cell antibodies in autoimmune cerebellar ataxia. Part 1: Anti-mGluR1, anti-Homer-3, anti-Sj/ITPR1 anti-CARP VIII. *J Neuroinflammation.* (2015) 12:166. doi: 10.1186/s12974-015-0356-y
91. Venkatraman A, Opal P. Paraneoplastic cerebellar degeneration with anti-Yo antibodies – a review. *Ann Clin Transl Neurol.* (2016) 3:655–63. doi: 10.1002/acn3.328
92. Drlicek M, Bianchi G, Bogliun G, Casati B, Grisold W, Kolig C, et al. Antibodies of the anti-Yo and anti-Ri type in the absence of paraneoplastic neurological syndromes: a long-term survey of ovarian cancer patients. *J Neurol.* (1997) 244:85–9. doi: 10.1007/s004150050054
93. Rana AQ, Rana AN, Adlul A. Acute ataxia due to anti-Yo antibody paraneoplastic cerebellar degeneration 4 months prior to diagnosis of uterine carcinoma. *Acta Neurol Belg.* (2012) 112:303–4. doi: 10.1007/s13760-012-0047-8

94. Hasadsri L, Lee J, Wang BH, Yekkirala L, Wang M. Anti-Yo associated paraneoplastic cerebellar degeneration in a man with large cell cancer of the lung. *Case Rep Neurol Med.* (2013) 2013:725936. doi: 10.1155/2013/725936
95. Bruhnding A, Notch D, Beard A. Anti-Yo positive paraneoplastic cerebellar degeneration in the setting of cholangiocarcinoma. *J Clin Neurosci.* (2017) 36:71–2. doi: 10.1016/j.jocn.2016.10.034
96. Corradi JP, Yang C, Darnell JC, Dalmau J, Darnell RB. A post-transcriptional regulatory mechanism restricts expression of the paraneoplastic cerebellar degeneration antigen cdr2 to immune privileged tissues. *J Neurosci.* (1997) 17:1406–15. doi: 10.1523/JNEUROSCI.17-04-01406.1997
97. Greenlee JE. Anti-Yo autoimmunity; dangerous for the brain but not the tumor? *J Neurol Sci.* (2006) 250:1–2. doi: 10.1016/j.jns.2006.08.001
98. Totland C, Aarskog NK, Eichler TW, Haugen M, Nøstbakken JK, Monstad SE, et al. CDR2 antigen and Yo antibodies. *Cancer Immunol Immunother.* (2011) 60:283–9. doi: 10.1007/s00262-010-0943-9
99. Raspotnig M, Haugen M, Thorsteinsdottir M, Stefansson I, Salvesen HB, Storstein A, et al. Cerebellar degeneration-related proteins 2 and 2-like are present in ovarian cancer in patients with and without Yo antibodies. *Cancer Immunol Immunother.* (2017) 66:1463–71. doi: 10.1007/s00262-017-2041-8
100. Okano HJ, Park W, Corradi JP, Darnell RB. The cytoplasmic Purkinje onconeural antigen cdr2 down-regulates c-Myc function : implications for neuronal and tumor cell survival. *Genes Dev.* (1999) 13:2087–97. doi: 10.1101/gad.13.16.2087
101. O'Donovan KJ, Diedler J, Couture GC, Fak JJ, Darnell RB. The onconeural antigen cdr2 is a novel APC / C target that acts in mitosis to regulate C-Myc target genes in mammalian tumor cells. *PLoS One.* (2010) 5:e10045. doi: 10.1371/journal.pone.0010045
102. Takanaga H, Mukai H, Shibata H, Toshimori M, Ono Y. PKN interacts with a paraneoplastic cerebellar degeneration-associated antigen, which is a potential transcription factor. *Exp Cell Res.* (1998) 241:363–72. doi: 10.1006/excr.1998.4060
103. Sakai K, Shirakawa T, Li Y, Kitagawa Y, Hirose G. Interaction of a paraneoplastic cerebellar degeneration-associated neuronal protein with the nuclear helix-loop-helix leucine zipper protein MRG X. *Mol Cell Neurosci.* (2002) 19:477–84. doi: 10.1006/mcne.2001.1059
104. Sakai K, Kitagawa Y, Saiki S, Saiki M, Hirose G. Effect of a paraneoplastic cerebellar degeneration-associated neural protein on B-myb promoter activity. *Neurobiol Dis.* (2004) 15:529–33. doi: 10.1016/j.nbd.2003.11.003
105. Sakai K, Kitagawa Y, Li Y, Shirakawa T, Hirose G. Suppression of the transcriptional activity and DNA binding of nuclear factor-kappa B by a paraneoplastic cerebellar degeneration-associated antigen. *J Neuroimmunol.* (2001) 119:10–5. doi: 10.1016/S0165-5728(01)00368-X
106. Kråkenes T, Herdlevær I, Raspotnig M, Haugen M, Schubert M, Vedeler CA. CDR2L is the major Yo antibody target in paraneoplastic cerebellar degeneration. *Ann Neurol.* (2019) 86:316–21. doi: 10.1002/ana.25511
107. Santomasso BD, Roberts WK, Thomas A, Williams T, Blachère NE, Dudley ME, et al. A T cell receptor associated with naturally occurring human tumor immunity. *Proc Natl Acad Sci USA.* (2007) 104:19073–8. doi: 10.1073/pnas.0704336104
108. Blachère NE, Orange DE, Santomasso BD, Doerner J, Foo PK, Herre M, et al. T cells targeting a neuronal paraneoplastic antigen mediate tumor rejection and trigger CNS autoimmunity with humoral activation. *Eur J Immunol.* (2014) 44:3240–51. doi: 10.1002/eji.201444624
109. Klein L, Kyewski B, Allen PM, Hogquist KA. Positive and negative selection of the T cell repertoire: what thymocytes see and don't see. *Nat Rev Immunol.* (2014) 14:377–91. doi: 10.1038/nri3667
110. Kondo K, Ohigashi I, Takahama Y. Thymus machinery for T-cell selection. *Int Immunol.* (2019) 31:119–25. doi: 10.1093/intimm/dxy081
111. Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, et al. Projection of an immunological self-shadow within the thymus by the aire protein. *Science.* (2002) 298:1395–401. doi: 10.1126/science.1075958
112. Malchow S, Leventhal DS, Lee V, Nishi S, Socci ND, Savage PA. Aire enforces immune tolerance by directing autoreactive T cells into the regulatory T cell lineage. *Immunity.* (2016) 44:1102–13. doi: 10.1016/j.immuni.2016.02.009
113. *Immunological Genome Project*. Available online at: http://www.immgen.org/databrowser/index.html?gene_symbol=CDR2 [accessed April 1, 2020].
114. Vialatte de Pémille C, Berzero G, Small M, Psimaras D, Giry M, Daniau M, et al. Transcriptomic immune profiling of ovarian cancers in paraneoplastic cerebellar degeneration associated with anti-Yo antibodies. *Br J Cancer.* (2018) 119:105–13. doi: 10.1038/s41416-018-0125-7
115. Yu W, Jiang N, Ebert PJR, Kidd BA, Müller S, Peder J, et al. Clonal deletion prunes, but does not eliminate self-specific αβ CD8+T lymphocytes. *Immunity.* (2015) 42:929–41. doi: 10.1016/j.immuni.2015.05.001
116. Carl PL, Temple BRS, Cohen PL. Most nuclear systemic autoantigens are extremely disordered proteins: implications for the etiology of systemic autoimmunity. *Arthritis Res Ther.* (2005) 7:R1360–74. doi: 10.1186/ar1832
117. Darnell JC, Albert ML, Darnell RB. cdr2, a target antigen of naturally occurring human tumor immunity, is widely expressed in gynecological tumors. *Cancer Res.* (2000) 60:2136–9.
118. Blachère NE, Orange DE, Gantman EC, Santomasso BD, Couture GC, Ramirez-Montagut T, et al. T cells presenting viral antigens or autoantigens induce cytotoxic T cell anergy. *JCI Insight.* (2017) 2:e96173. doi: 10.1172/jci.insight.96173
119. Small M, Treilleux I, Couillault C, Pissaloux D, Picard G, Paindavoine S, et al. Genetic alterations and tumor immune attack in Yo paraneoplastic cerebellar degeneration. *Acta Neuropathol.* (2018) 135:569–79. doi: 10.1007/s00401-017-1802-y
120. Movassagh M, Spatz A, Davoust J, Lebecque S, Romero P, Pittet M, et al. Selective accumulation of mature DC-lamp+ dendritic cells in tumor sites is associated with efficient T-cell-mediated antitumor response and control of metastatic dissemination in melanoma. *Cancer Res.* (2004) 64:2192–8. doi: 10.1158/0008-5472.CAN-03-2969
121. Engelhardt B, Ransohoff RM. Capture, crawl, cross: the T cell code to breach the blood-brain barriers. *Trends Immunol.* (2012) 33:579–89. doi: 10.1016/j.it.2012.07.004
122. Kerfoot SM, Kubes P. Overlapping roles of P-selectin and α 4 integrin to recruit leukocytes to the central nervous system in experimental autoimmune encephalomyelitis. *J Immunol.* (2002) 169:1000–6. doi: 10.4049/jimmunol.169.2.1000
123. Vajkoczy P, Laschinger M, Engelhardt B. α4-integrin-VCAM-1 binding mediates G protein-independent capture of encephalitogenic T cell blasts to CNS white matter microvessels. *J Clin Invest.* (2001) 108:557–65. doi: 10.1172/JCI12440
124. Bauer M, Brakebusch C, Coisne C, Sixt M, Wekerle H, Engelhardt B, et al. β1 integrins differentially control extravasation of inflammatory cell subsets into the CNS during autoimmunity. *Proc Natl Acad Sci USA.* (2009) 106:1920–5. doi: 10.1073/pnas.0808909106
125. Holman DW, Klein RS, Ransohoff RM. The blood-brain barrier, chemokines and multiple sclerosis. *Biochim Biophys Acta Mol Basis Dis.* (2011) 1812:220–30. doi: 10.1016/j.bbadis.2010.07.019
126. Ransohoff RM, Engelhardt B. The anatomical and cellular basis of immune surveillance in the central nervous system. *Nat Rev Immunol.* (2012) 12:623–35. doi: 10.1038/nri3265
127. Battistini L, Piccio L, Rossi B, Bach S, Galgani S, Gasperini C, et al. CD8+ T cells from patients with acute multiple sclerosis display selective increase of adhesiveness in brain venules: a critical role for P-selectin glycoprotein ligand-1. *Blood.* (2003) 101:4775–82. doi: 10.1182/blood-2002-10-3309
128. Ifergan I, Kebir H, Alvarez JI, Marceau G, Bernard M, Bourbonnire L, et al. Central nervous system recruitment of effector memory CD8+ T lymphocytes during neuroinflammation is dependent on α4 integrin. *Brain.* (2011) 134:3557–74. doi: 10.1093/brain/awr268
129. Martin-Blondel G, Pignolet B, Tietz S, Yshii L, Gebauer C, Perinat T, et al. Migration of encephalitogenic CD8 T cells into the central nervous system is dependent on the α4β1-integrin. *Eur J Immunol.* (2015) 45:3302–12. doi: 10.1002/eji.201545632
130. Hottinger AF, de Micheli R, Guido V, Karampera A, Hagmann P, Du Pasquier R. Natalizumab may control immune checkpoint inhibitor-induced limbic encephalitis. *Neurol Neuroimmunol NeuroInflamm.* (2018) 5:e439. doi: 10.1212/NXI.0000000000000439
131. Gross CC, Meyer C, Bhatia U, Yshii L, Kleffner I, Bauer J, et al. CD8+ T cell-mediated endotheliopathy is a targetable mechanism of neuro-inflammation in Susac syndrome. *Nat Commun.* (2019) 10:5779. doi: 10.1038/s41467-019-13593-5
132. Wilhelm I, Nyúl-Tóth Á, Suciu M, Hermenean A, Krizbai IA. Heterogeneity of the blood-brain barrier. *Tissue Barriers.* (2016) 4:e1143544. doi: 10.1080/21688370.2016.1143544

133. Schinkel AH, Wagenaar E, van Deemter L, Mol CAAM, Borst P. Absence of the mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. *J Clin Invest.* (1995) 96:1698–705. doi: 10.1172/JCI118214
134. Yasuda K, Cline C, Lin YS, Scheib R, Ganguly S, Thirumaran RK, et al. *In vivo* imaging of human MDR1 transcription in the brain and spine of MDR1-luciferase reporter mice. *Drug Metab Dispos.* (2015) 43:1646–54. doi: 10.1124/dmd.115.065078
135. Verschuuren J, Chuang L, Rosenblum MK, Lieberman F, Pryor A, Posner JB, et al. Inflammatory infiltrates and complete absence of Purkinje cells in anti-Yo-associated paraneoplastic cerebellar degeneration. *Acta Neuropathol.* (1996) 91:519–25. doi: 10.1007/s004010050460
136. Greenlee JE, Brashear RH. Antibodies to cerebellar Purkmje cells in patients with paraneoplastic cerebellar degeneration and ovarian Carcinoma. *Ann Neurol.* (1983) 14:609–13. doi: 10.1002/ana.410140603
137. Giometto B, Marchiori GC, Nicolao P, Scaravilli T, Lion A, Bardin PG, et al. Sub-acute cerebellar degeneration with anti-Yo autoantibodies: immunohistochemical analysis of the immune reaction in the central nervous system. *Neuropathol Appl Neurobiol.* (1997) 23:468–74. doi: 10.1046/j.1365-2990.1997.00078.x
138. Storstein A, Krossnes BK, Vedeler CA. Morphological and immunohistochemical characterization of paraneoplastic cerebellar degeneration associated with yo antibodies. *Acta Neurol Scand.* (2009) 120:64–7. doi: 10.1111/j.1600-0404.2008.01138.x
139. Aye MM, Kasai T, Tashiro Y, Xing HQ, Shirahama H, Mitsuda M, et al. CD8 positive T-cell infiltration in the dentate nucleus of paraneoplastic cerebellar degeneration. *J Neuroimmunol.* (2009) 208:136–40. doi: 10.1016/j.jneuroim.2009.01.017
140. Aboul-Enein F, Höftberger R, Buxhofer-Ausch V, Drlicek M, Lassmann H, Budka H, et al. Neocortical neurones may be targeted by immune attack in anti-Yo paraneoplastic syndrome. *Neuropathol Appl Neurobiol.* (2008) 34:248–52. doi: 10.1111/j.1365-2990.2007.00909.x
141. Bien CG, Vincent A, Barnett MH, Becker AJ, Blümcke I, Graus F, et al. Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. *Brain.* (2012) 135:1622–38. doi: 10.1093/brain/aws082
142. McConnell MJ, Huang YH, Datwani A, Shatz CJ. H2-K b and H2-D b regulate cerebellar long-term depression and limit motor learning. *Proc Natl Acad Sci USA.* (2009) 106:6784–9. doi: 10.1073/pnas.0902018106
143. Yshii L, Pignolet B, Mauré E, Pierau M, Brunner-Weinzierl M, Hartley O, et al. IFN-γ is a therapeutic target in paraneoplastic cerebellar degeneration. *JCI Insight.* (2019) 4:127001. doi: 10.1172/jci.insight.127001
144. Pellkofer H, Schubart AS, Höftberger R, Schutze N, Pagany M, Schüller M, et al. Modelling paraneoplastic CNS disease: T-cells specific for the onconeuronal antigen PNMA1 mediate autoimmune encephalomyelitis in the rat. *Brain.* (2004) 127:1822–30. doi: 10.1093/brain/awh205
145. Gebauer C, Pignolet B, Yshii L, Mauré E, Bauer J, Liblau R. CD4+ and CD8+ T cells are both needed to induce paraneoplastic neurological disease in a mouse model. *Oncoimmunology.* (2017) 6:e1260212. doi: 10.1080/2162402X.2016.1260212
146. Yshii LM, Gebauer CM, Pignolet B, Mauré E, Quériault C, Pierau M, et al. CTLA4 blockade elicits paraneoplastic neurological disease in a mouse model. *Brain.* (2016) 139:2923–34. doi: 10.1093/brain/aww225
147. Greenlee JE, Parks T, Jaeckle K. Type IIa ('anti-Hu') antineuronal antibodies produce destruction of rat cerebellar granule neurons *in vitro*. *Neurology.* (1993) 43:2049–54. doi: 10.1212/WNL.43.10.2049
148. Darnell RB. Onconeural antigens and the paraneoplastic neurologic disorders: At the intersection of cancer, immunity, and the brain. *Proc Natl Acad Sci USA.* (1996) 93:4529–36. doi: 10.1073/pnas.93.10.4529
149. Tanaka K, Tanaka M, Igarashi S, Onodera O, Miyatake T, Tsuji S. Trial to establish an animal model of paraneoplastic cerebellar degeneration with anti-Yo antibody. 2. Passive transfer of murine mononuclear cells activated with recombinant Yo protein to paraneoplastic cerebellar degeneration lymphocytes in severe combi. *Clin Neurol Neurosurg.* (1995) 97:101–5. doi: 10.1016/0303-8467(95)00005-5
150. Smitt S, Manley GT, Posner JB. Immunization with the paraneoplastic encephalomyelitis antigen hud does not cause neurologic disease in mice. *Neurology.* (1995) 45:1873–8. doi: 10.1212/WNL.45.10.1873
151. Carpentier AF, Rosenfeld MR, Delattre JY, Whalen RG, Posner JB, Dalmau J. DNA vaccination with HuD inhibits growth of a neuroblastoma in mice. *Clin Cancer Res.* (1998) 4:2819–24.
152. Panja D, Vedeler CA, Schubert M. Paraneoplastic cerebellar degeneration: Yo antibody alters mitochondrial calcium buffering capacity. *Neuropathol Appl Neurobiol.* (2019) 45:141–56. doi: 10.1111/nan.12492
153. Greenlee JE, Clawson SA, Hill KE, Wood B, Clardy SL, Tsunoda I, et al. Anti-Yo antibody uptake and interaction with its intracellular target antigen causes Purkinje cell death in rat cerebellar slice cultures: a possible mechanism for paraneoplastic cerebellar degeneration in humans with gynecological or breast cancers. *PLoS One.* (2015) 10:e0123446.doi: 10.1371/journal.pone.0123446
154. de Giorgio R, Bovara M, Barbara G, Canossa M, Sarnelli G, de Ponti F, et al. Anti-HuD-induced neuronal apoptosis underlying paraneoplastic gut dysmotility. *Gastroenterology.* (2003) 125:70–9. doi: 10.1016/S0016-5085(03)00664-4
155. Greenlee JE, Clawson SA, Hill KE, Wood BL, Tsunoda I, Carlson NG. Purkinje cell death after uptake of anti-yo antibodies in cerebellar slice cultures. *J Neuropathol Exp Neurol.* (2010) 69:997–1007. doi: 10.1097/NEN.0b013e3181f0c82b
156. Yshii LM, Hohlfeld R, Liblau RS. Inflammatory CNS disease caused by immune checkpoint inhibitors: status and perspectives. *Nat Rev Neurol.* (2017) 13:755–63. doi: 10.1038/nrneurol.2017.144
157. Vogrig A, Fouret M, Joubert B, Picard G, Rogemond V, Pinto AL, et al. Increased frequency of anti-Ma2 encephalitis associated with immune checkpoint inhibitors. *Neurol Neuroimmunol NeuroInflamm.* (2019) 6:e604. doi: 10.1212/NXI.0000000000000604
158. Williams TJ, Benavides DR, Patrice K-A, Dalmau JO, de Ávila ALR, Le DT, et al. Association of autoimmune encephalitis with combined immune checkpoint inhibitor treatment for metastatic cancer. *JAMA Neurol.* (2016) 73:928. doi: 10.1001/jamaneurol.2016.1399
159. Larkin J, Chmielowski B, Lao CD, Hodi FS, Sharfman W, Weber J, et al. Neurologic serious adverse events associated with nivolumab plus ipilimumab or nivolumab alone in advanced melanoma, including a case series of encephalitis. *Oncologist.* (2017) 22:709–18. doi: 10.1634/theoncologist.2016-0487
160. Wei SC, Levine JH, Cogdill AP, Zhao Y, Anang NAS, Andrews MC, et al. Distinct cellular mechanisms underlie anti-CTLA-4 and anti-PD-1 checkpoint blockade. *Cell.* (2017) 170:1120–33. doi: 10.1016/j.cell.2017.07.024
161. Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. *Nat Rev Cancer.* (2019) 19:307–25. doi: 10.1038/s41568-019-0144-6
162. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, et al. B cells and tertiary lymphoid structures promote immunotherapy response. *Nature.* (2020) 577:549–55. doi: 10.1038/s41586-019-1922-8
163. Joseph CG, Darrah E, Shah AA, Skora AD, Livia A, Wigley FM, et al. Association of the autoimmune disease scleroderma with an immunologic response to cancer. *Science.* (2014) 343:152–7. doi: 10.1126/science.1246886
164. Albert ML, Austin LM, Darnell RB. Detection and treatment of activated T cells in the cerebrospinal fluid of patients with paraneoplastic cerebellar degeneration. *Ann Neurol.* (2000) 47:9–17. doi: 10.1002/1531-8249(200001)47:1<9::AID-ANA5>3.0.CO;2-I
165. Beltrán E, Gerdes LA, Hansen J, Flierl-Hecht A, Krebs S, Blum H, et al. Early adaptive immune activation detected in monozygotic twins with prodromal multiple sclerosis. *J Clin Invest.* (2019) 129:4758–68. doi: 10.1172/JCI128475
166. Ahrends T, Spanjaard A, Pilzecker B, Babała N, Bovens A, Xiao Y, et al. CD4+ T cell help confers a cytotoxic T cell effector program including coinhibitory receptor downregulation and increased tissue invasiveness. *Immunity.* (2017) 47:848–61.e5. doi: 10.1016/j.immuni.2017.10.009
167. Roberts WK, Blachère NE, Frank MO, Dousmanis A, Ransohoff RM, Darnell RB. A destructive feedback loop mediated by CXCL10 in central nervous system inflammatory disease. *Ann Neurol.* (2015) 78:619–29. doi: 10.1002/ana.24494
168. Di Liberto G, Pantelyushin S, Kreutzfeldt M, Page N, Musardo S, Coras R, et al. Neurons under T cell attack coordinate phagocyte-mediated synaptic stripping. *Cell.* (2018) 175:458–71.e19. doi: 10.1016/j.cell.2018.07.049

169. Neumann H, Cavalié A, Jenne D, Wekerle H. Major Histocompatibility Complex (MHC) Class I gene expression in singe neurons of the central nervous system : differential regulationby interferon (IFN)-gamma and tumor necrosis factor (TNF)-alpha. *J Exp Med.* (1997) 185:305–16. doi: 10.1084/jem.185.2.305
170. Tröscher AR, Wimmer I, Quemada-Garrido L, Köck U, Gessl D, Verberk SGS, et al. Microglial nodules provide the environment for pathogenic T cells in human encephalitis. *Acta Neuropathol.* (2019) 137:619–35. doi: 10.1007/s00401-019-01958-5
171. Galli J, Greenlee J. Paraneoplastic diseases of the central nervous system. *Lancet Neurol.* (2020) 9:167. doi: 10.12688/f1000research.21309.1
172. Flach AC, Litke T, Strauss J, Haberl M, Cordero Gómez C, Reindl M, et al. Autoantibody-boosted T-cell reactivation in the target organ triggers manifestation of autoimmune CNS disease. *Proc Natl Acad Sci USA.* (2016) 113:3323–8. doi: 10.1073/pnas.1519608113
173. Meloni C, Iani C, Dominijanni S, Arciprete F, Cipriani S, Caramiello MS, et al. A case report of plasma exchange therapy in non-paraneoplastic cerebellar ataxia associated with Anti-Yo antibody. *Ther Apher Dial.* (2004) 8:500–2. doi: 10.1111/j.1774-9987.2004.00198.x
174. Thöne J, Hohaus A, Lamprecht S, Bickel A, Erbguth F. Effective immunosuppressant therapy with cyclophosphamide and corticosteroids in paraneoplastic cerebellar degeneration. *J Neurol Sci.* (2008) 272:171–3. doi: 10.1016/j.jns.2008.04.020
175. Shams'ili S, de Beukelaar J, Gratama JW, Hooijkaas H, van Den Bent M, van't Veer M, et al. An uncontrolled trial of rituximab for antibody associated paraneoplastic neurological syndromes. *J Neurol.* (2006) 253:16–20. doi: 10.1007/s00415-005-0882-0
176. Rojas I, Graus F, Keime-Guibert F, Reñé R, Delattre JY, Ramón JM, et al. Long-term clinical outcome of paraneoplastic cerebellar degeneration and anti-Yo antibodies. *Neurology.* (2000) 55:713–5. doi: 10.1212/WNL.55.5.713
177. Schessl J, Schuberth M, Reilich P, Schneiderat P, Strigl-Pill N, Walter MC, et al. Long-term efficiency of intravenously administered immunoglobulin in anti-yo syndrome with paraneoplastic cerebellar degeneration. *J Neurol.* (2011) 258:946–7. doi: 10.1007/s00415-010-5859-y
178. Phuphanich S, Brock C. Neurologic improvement after high-dose intravenous immunoglobulin therapy in patients with paraneoplastic cerebellar degeneration associated with anti-purkinje cell antibody. *J Neurooncol.* (2007) 81:67–9. doi: 10.1007/s11060-006-9198-x
179. Baker SK, Morillo C, Vernino S. Autoimmune autonomic ganglionopathy with late-onset encephalopathy. *Auton Neurosci Basic Clin.* (2009) 146:29–32. doi: 10.1016/j.autneu.2008.10.016
180. Daver N, McClain K, Allen CE, Parikh SA, Otrock Z, Rojas-Hernandez C, et al. A consensus review on malignancy-associated hemophagocytic lymphohistiocytosis in adults. *Cancer.* (2017) 123:3229–40. doi: 10.1002/cncr.30826

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