Case Report

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Title: A complex and severe encephalitis associated with four co-existing cell-surface autoantibodies

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Abstract

Background and objectives:
Many forms of autoimmune encephalitis are mediated by cell-surface directed autoantibodies. Typically one autoantibody specificity is found per patient. The co-occurrence of four cell-surface antibodies in a single patient is exceptionally rare. We report a patient who had a complex and severe encephalitis associated with antibodies to NMDA, Glycine, $GABA_A$ and $GABA_B$ receptors.

Methods: n/a

Results:
A 28-year-old man on tacrolimus after liver transplantation presented with a first seizure following a gastrointestinal illness. Over a few weeks, he developed confusion, cerebellar signs, opsoclonus, neuromyotonia and medication-refractory seizures. CSF sampling revealed 826 white cells and NMDA, glycine and $GABA_B$ receptor antibodies: all were also detected in serum along with additional $GABA_A$ receptor antibodies. MRI brain demonstrated cerebellar leptomeningeal enhancement and a hyperintense lesion in the cerebellar vermis. EEG revealed extreme delta brush and needle EMG confirmed neuromyotonia . No underlying malignancy was detected. Methylprednisolone, IVIG, Rituximab, therapeutic plasma exchange, cyclophosphamide and bortezomib were administered sequentially, with minimal clinical improvement. Death secondary to respiratory sepsis occurred on the 714^{th} hospital day. Postmortem revealed pan-cerebellar atrophy with Purkinje cell loss; dentate nucleus ganglionopathy with mononuclear and CD8+ T cell dominant infiltrates, and thoracolumbar cord myelopathy.

Conclusion: The detection of multiple cell-surface antibodies in autoimmune encephalitis is unusual and may result in complex overlap syndromes with a poor response to immunotherapy.

Case

A 28-year-old right-handed man presented with a generalised seizure lasting 4 minutes, headaches and pyrexia one week following a diarrheal illness contracted while abroad in the United States. He had been maintained on tacrolimus since an orthotopic liver transplant in 2007 (aged 19) for liver cirrhosis secondary to α1 anti-trypsin deficiency. Neurological examination at first presentation was normal. Routine serology in addition to an extensive screen for infectious causes were normal (**Supplementary table 1**). CSF sampling revealed a pleocytosis with 826 white cells per cm^3 (95% lymphocytes) and a protein of 1.03 g/L. He was treated with antiviral and antibacterial therapy for presumed infective encephalitis. Over the subsequent two weeks, further generalised seizures were accompanied by confusion, cerebellar ataxia and muscle twitching. Now, neurologic examination revealed multidirectional nystagmus, past pointing and dysdiadochokinesis in addition to pyramidal signs which included ankle clonus and an extensor plantar response. There was further clinical deterioration with onset of frequent anti-seizure medication-refractory seizures. Within one week, he became obtunded and developed myoclonus, opsoclonus, autonomic dysfunction, generalised rigidity and fasciculations in the upper and lower limbs.

MRI brain revealed subtle T1 post-contrast leptomeningeal enhancement in the cerebellum and a solitary T2 hyperintensity in the cerebellar vermis, with subsequent development of generalised cerebral atrophy over months **(Figure 1)**. CT thorax abdomen and pelvis and full-body PET CT revealed no malignancy. Electroencephalogram demonstrated initially sharp and slow wave activity in the right anterior temporal regions and periodic discharges in the temporal regions bilaterally, and showed extreme delta brush as the patient deteriorated clinically **(Figure 2)**. Electromyography revealed peripheral nerve hyperexcitability with neuromyotonia in the right tibialis anterior and right medial gastrocnemius **(Figure 2)**. Serum autoimmune encephalitis panel testing returned positive antibodies to NMDA, Glycine, $GABA_A$ and $GABA_B$ receptors (performed at Oxford Immunology Laboratory, Oxford, United Kingdom) with detectable NMDA- (titre 1:10), Glycine- (titre 1:100) and $GABA_B$ (titre 1:50) – receptor antibodies in paired CSF. Other autoantibodies were negative (**Supplementary table 1**). The patient was treated with high dose intravenous methylprednisolone 36 days following presentation. This led to no improvement, prompting intravenous immunoglobulin (IVIG), and subsequently, 5 x 5-7 day courses of therapeutic plasma exchange. Still given no clinical improvement, rituximab was initiated but resulted in minimal clinical and electrographic improvement. Thereafter, cyclophosphamide and bortezomib were trialled

with no clinical change. A timeline of treatment is provided in **Supplementary figure 1**. Unfortunately, death secondary to a respiratory tract infection (aggressive treatment not pursued) occurred on the 714th hospital day. Post-mortem studies revealed pan-cerebellar atrophy with Purkinje cell loss, a florid dentate ganglionopathy with numerous CD68 positive macrophages, rod-shaped microglial cells and sparse CD8^{+} T cells. No B cells were observed. There was also evidence of myelopathy involving the ventral and lateral corticospinal tracts together with the fasciculus gracilis which involved the entire spinal cord. The cerebral cortex was macroscopically and microscopically normal at time of autopsy. **(Figure 3)**

Discussion

Autoantibody co-occurrences

We report an unusual case of AE with four co-existing antibodies all directed against cell-surface antigens in a patient on long-term immunosuppression. Autoantibody-positive AE is characteristically associated with the identification of single rather than multiple IgG antibody biomarkers in an individual patient.[1] When multiple autoantibodies co-occur, they most frequently target intracellular antigens, rather than surface antigens, and confer a higher risk of underlying malignancy than cases defined by a single antibody.[2,3] However, among antibodies to cell-surface antigens notable exceptions exist such as the well-described co-occurrence of LGI1 and CASPR2 antibodies.[4,5] $GABA_B$ receptor autoimmunity may co-occur with other antibodies in up to 35% of cases (usually antibodies to intracellular targets but it has also been reported to co-exist with NMDA-R antibodies).[6] In a recent study of AMPA-R autoimmunity, 60% of patients had co-existing antibodies, most frequently ACh-R IgG (40%) but also anti-VGCC (P/Q- and N-type, 24%), NMDA-R (12%), CASPR2 (3%), LGI1 (2%) autoantibodies.[7] The co-occurrence of antibodies to NMDA- and glycine receptors has been previously described in a young man with a similar clinical syndrome to our case which also resulted in a fatal outcome.[8] A table of reported co-existing cell-surface directed antibodies can be found in **Supplementary Table 2.** In our case the detection of four cell-surface-directed antibodies in a single patient, each potentially contributing to the clinical syndrome, is an exceptionally rare phenomenon. Two important questions raised by this case are i) whether multiple cell surface antibodies led to an 'overlap' syndrome, due to the simultaneous targeting of multiple nervous system antigens and ii) if this combination resulted in the lack of immunotherapy response.

Clinical syndrome

Clinical 'overlap' syndromes resulting from the co-occurrence of multiple cell-surface-

directed antibodies have been described in ACh-R-ab-positive myasthenia gravis co-occurring with AQP4-ab-positive neuromyelitis optica spectrum disorder.[9] Similarly, patients with NMDA-R encephalitis and CNS demyelination associated with MOG-IgG and AQP4-IgG have been reported.[10] These syndromes can have distinctive features compared to cases defined by a single antibody: for example, ACh-R-positive myasthenia gravis co-occurring with NMOSD typically follows a benign clinical course, and LGI1 + CASPR2-autoimmunity is more likely to result in Morvan's syndrome and to be associated with underlying thymoma than when either of these antibodies occur in isolation.[4,5] Similarly, in this patient, certain clinical and paraclinical features are evocative of specific antibody-associated neurological disorders such as the presence of hyperekplexia, limb rigidity and myoclonus (most consistent with glycine receptor antibody autoimmunity), opsoclonus-myoclonus (observed in $GABA_B$-, NMDA- and glycine receptor autoimmunity), autonomic dysfunction and the extreme delta brush sign on EEG (NMDA-R encephalitis) and cerebellar ataxia (NMDA- and $GABA_B$-receptor autoimmunity).[6,11–18] While encephalopathy and refractory seizures may occur in any of the above disorders, they are characteristic of $GABA_A$ and $GABA_B$ receptor antibody autoimmunity.[12,19] An unusual aspect of this case is the presence of neuromyotonia on needle EMG which, in an autoimmune context, has a strong association with antibodies to CASPR2 or, less commonly, LGI1.[20] In this case neither antibody was detected despite repeated testing, raising the possibility that this patient harboured an additional unclassified autoantibody (or was missed on testing).

Outcome

The poor treatment response in this case is notable as syndromes associated with cell-surface antibodies are typically immunotherapy responsive. With prompt initiation of immunotherapy, up to 81% of patients with NMDA-R antibody encephalitis have a modified Rankin score (mRS) of <2 at 24 months; the median mRS was 1 for patients with glycine receptor antibody syndromes on long-term follow-up.[15,21] Similarly, $GABA_AR$ and $GABA_BR$ antibody encephalitides both respond to immunotherapy in over 80% of cases.[12,19] The presence of the extreme delta brush sign on EEG in this case was a late electrographic finding, first appearing 6 months following initial presentation. In NMDA-R encephalitis this feature may be associated with more severe disease.[11] A differentiated response to immunotherapy has been observed in NMDA-R encephalitis depending on whether AQP4 or MOG antibodies are also present, indicating that various cell-surface antibody combinations may inform prognosis and treatment response.[22] In this case, the presence of four cell-surface antibodies likely resulted in a complex autoimmune neurological disorder which did not respond to immunotherapy and ultimately had a fatal outcome. A similar case of NMDA-

R and Glycine-R antibodies co-occurrence reported in the literature also responded poorly to immunotherapy, resulting in a fatal outcome, suggesting that the presence of multiple cell-surface antibodies may confer a starker prognosis than cases defined by a single antibody. [8]

Another association of multiple autoantibodies is malignancy. However, no cancer was found in this case despite extensive investigation. Nevertheless, paraneoplastic neurologic disorders can precede cancer detection by up to 5 years, and the presence of $GABA_B$ receptor antibodies in particular carries a >50% risk for underlying malignancy (usually small cell lung cancer).[12,28]

Autopsy

Postmortem studies in patients with autoimmune encephalopathies are relatively few given the novelty of these disorders and the typical immunotherapy-responsiveness of syndromes associated with cell-surface antibodies.[23] In this case abnormal pathological findings included loss of Purkinje cells and dentate ganglionopathy, consistent with previously reported autopsy cases of paraneoplastic cerebellar syndromes.[24,25] In contrast to a similar fatal case of NMDA- and Glycine receptor autoimmunity, there was no evidence of active cortical inflammation in this case, possibly due to the long latency between symptom onset and death (almost 2 years).[8] A surprising feature of this case was the widespread tract-specific myelopathy without active inflammation at the time of autopsy. This is distinct from reported pathological findings in PERM of neuronal loss in the anterior horn cells, perivascular lymphocyte cuffs and microglial nodules.[26] Paraneoplastic myelopathies such as those associated with ANNA-2 antibodies have been reported with tract-specific spinal cord findings at the time of autopsy.[27]

Conclusion

The co-occurrence of four potentially pathogenic antibodies is a rare phenomenon in autoimmune encephalitis and may result in a more complex phenotype than cases defined by a single antibody. The prognosis and optimal treatment regimen for autoimmune encephalitis with multiple antibodies is poorly understood and represents a field of future study.

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Figures: title and legends

Figure 1: A) T1 post-contrast MRI showing subtle leptomeningeal enhancement within the cerebellum (arrow) **B)** T2 FLAIR hyperintensity within the cerebellar vermis (arrow) and generalised cerebral atrophy.

Figure 2: A) Electroencephalogram (EEG) of 28-year-old male, unresponsive state. International 10-20 system of electrode placement and left-over-right bipolar montage; ECG: electrocardiogram; EOC: electrooculogram; µV: microvolt; Hz: hertz. Tracing demonstrates frequent delta waves with superimposed bursts of beta activity known as the 'extreme delta brush' sign (arrow). **B)** Electromyogram (EMG) of right tibialis anterior in 28-year-old male. mV: microvolt; msec: millisecond. Tracing demonstrates frequent low-amplitude fasciculations and neuromyotonia (arrow) with an intraburst frequency of up to 800Hz.

Figure 3: A) Cerebellar Dentate nucleus showing sparse infiltrate of CD3 Positive T-Lymphocytes. No B cells were observed. B) Dentate nucleus showing heavy infiltrate of CD68 positive macrophages. C) High power view of cerebellar dentate neurons showing mononuclear infiltrate and rod shape microglial cells. D) Cerebellar cortex remote from vermis showing complete absence of Purkinje Cells with Bergmann gliosis at interface between molecular and granular layers. E) Cerebellar dentate nucleus showing extreme hypercellularity with vacuolar change in hilum.