Seronegative antibody-mediated neurology after immune checkpoint inhibitors

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Abstract

Checkpoint-inhibitor medications have revolutionized oncology practice, but frequently induce immune-related adverse events. During autoimmune neurology practice over 20-months, we prospectively identified four patients with likely antibody-mediated neurological diseases after checkpoint-inhibitors: longitudinally extensive transverse myelitis, Guillain-Barré syndrome and myasthenia gravis. All patients shared three characteristics: symptoms commenced four-weeks after drug administration, responses to conventional immunotherapies were excellent and autoantibodies traditionally associated with their syndrome were absent. Indeed, serum immunoglobulins from the myelitis and Guillain-Barré syndrome patients showed novel patterns of tissue reactivity. Vigilance is required for antibody-mediated neurology after checkpoint-inhibitor administration. This phenomenon may inform the immunobiology of antibody-mediated diseases.

Introduction

A major recent advance in oncology has been the success of T cell checkpoint-inhibitors. These drugs are increasingly used to treat various cancers including melanoma, kidney adenocarcinoma, lung carcinomas, and hematological malignancies.[1] They commonly target programmed death 1 (PD-1) or cytotoxic T lymphocyte antigen-4 (CTLA-4) and, more rarely, PD-ligand 1 (PD-L1). PD-1 and CTLA-4 are expressed in both conventional T cells and regulatory T cells (Tregs), amongst other cell types.[2,3] However, immune-relatedadverse-events (irAEs) affect up to 40% of patients treated with checkpoint inhibitors and include colitis, dermatitis, pneumonitis, and hepatitis.[4] More rarely, neurological side-effects are observed: the most frequent is hypophysitis.[5] While conventional models typically suggest irAEs are T cell mediated, here, we describe likely antibody-mediated autoimmunity with the first case of immune checkpoint-blockade associated with longitudinally extensive transverse myelitis (LETM, n=1), and cases who developed myasthenia gravis (MG, n=2) and Guillain-Barre syndrome (GBS, n=1) after checkpoint inhibitors. The escalating use of these drugs in oncology requires heightened vigilance amongst neurologists for these associated, often seronegative, autoantibody-mediated side-effects that respond well to conventional immunotherapies.

Patients and Methods

Four patients prospectively observed during routine Autoimmune Neurology practice between July 2015 and March 2017 administered immune checkpoint-inhibitors are summarized in Table 1. We performed blinded testing of serum for autoantibodies in all patients, almost exclusively using previously described live cell based assay methodologies (Table 1),[6,7]and flow cytometry from whole blood in patient 1 . Written informed consent was obtained with ethical approval (REC16/YH/0013).

Results

Clinical features

Patient 1 was a 35-year-old male diagnosed in 2005 with stage IIa Hodgkin lymphoma which relapsed despite three lines of chemotherapy over 10 years (Table 1). Residual metastases prompted initiation of pembrolizumab (a humanized PD-1 monoclonal antibody) as 4th line therapy, with two cycles given at three week intervals. One week after the second cycle, he developed acute urinary retention, constipation, hiccoughs and vomiting, with weakness and sensory loss in arms and legs. Examination revealed a spastic tetraparesis with profound sensory loss and sphincter atonia. MRI showed a LETM from the pons to the lower thoracic spine with extensive cord edema (Figure 1A-B). Aquaporin-4 and myelin-oligodendrocyte glycoprotein antibodies were not detected. CSF showed 24 mononuclear cells/mm^3; other detailed CSF and blood tests were unremarkable (Supplementary Table 1). Intravenous methylprednisolone and, subsequently, plasma exchange were administered with the aim of depleting free circulating pembrolizumab and any putative autoantibody. During an oral prednisolone taper, repeat MRI at six months showed considerable reduction of edema (Figure 1C-D). One year from symptom onset, he was continent, independent in ambulation, and his Hodgkin lymphoma had responded to the treatment.

To explore the underlying immunology, flow cytometry was performed from the patient's peripheral blood mononuclear cells at the nadir of the neurological syndrome. This revealed a decrease in Treg numbers ($CD3^{+}CD4^{+}CD25^{+}CD127^{lo}$; Figure 1E-F) and an anti-human-IgG antibody preferentially bound to the residual Tregs that expressed the highest levels of CD62L and CD25, indicating a sub-population targeted by the humanized pembrolizumab (Figure 1G-H).

Patient 2 was a 57-year-old male with metastatic melanoma involving the left axilla, mediastinum and lung hilum. Four weeks after the first infusion of nivolumab (a humanized PD-1 monoclonal antibody; at 1mg/kg) and ipilimumab (a CTLA-4 blocking antibody; at 3mg/kg), and one week after the second course, he developed exertional breathlessness with diplopia and ptosis which worsened towards the end of the day. Examination showed weak left eye abduction, right eye adduction and bilateral asymmetrical fatigable ptosis. The remainder of the examination and investigations were unremarkable. A clinical diagnosis of MG prompted intravenous methylprednisolone followed by 80mg oral prednisolone, with a rapid improvement in all features. All known MG-associated autoantibodies were negative (Table 1). The corticosteroids were gradually tapered. Patient 3 also developed seronegative MG, with limb weakness and ptosis, four weeks after the first dose of pembrolizumab for metastatic lung adenocarcinoma. Complete remission was achieved three months after commencing pyridostigmine and corticosteroids.

Patient 4 was a 52-year-old male with metastatic melanoma affecting the ear, cervical lymph nodes and lung. Treatments included radical neck dissection, and nivolumab with ipilimumab. Three weeks after the first infusion, and two days after the second cycle, he developed headache and generalized tiredness. Investigations revealed hypophysitis and prednisolone was commenced. A week later, he experienced progressive hand and feet numbness, with bilateral facial weakness, distal limb weakness and reduced deep tendon reflexes. CSF was acellular with a protein content of 2.3 g/L. Neurophysiology revealed a demyelinating neuropathy and both whole spine MRI and ganglioside antibodies were unremarkable (Table 1). In summary, findings were consistent with the acute inflammatory demyelinating polyneuropathy variant of GBS. He was commenced on intravenous immunoglobulins with a very good recovery.

In all four patients, the checkpoint inhibitors were discontinued and no neurological relapses were noted at follow-up (0.5-2.5 years, Table 1).

Over these 20 months, autoimmune neurology cases secondary to checkpoint inhibitors seen by the authors exceeded the cumulative frequency of patients with antibodies against dipeptidyl-peptidase-like protein-6 (DPPX, n=1), the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor receptor (AMPAR, n=0), the gamma-aminobutyric acid A-receptor ($GABA_AR$, n=1) and Iglon5 (n=0).[8]

Novel autoantibody detection

Serum immunoglobulin G exclusively from the patient with LETM bound to rodent brain tissue in a pattern comparable to aquaporin-4 (Figure 2A-C). Similarly, serum immunoglobulin M from the GBS patient, but not the other three patients, bound myelinating co-cultured induced pluripotent stem cell derived sensory neurons and rat primary Schwann cells (Figure 2D), prepared as previously described.[9] These reactivities were not seen in 20 healthy controls. No patient immunoglobulins bound to rodent muscle sections, C2C12 myotubes or CN21 muscle cell lines.

Discussion

Immune checkpoint-inhibitors provide an increasingly popular and contemporary approachto effective treatment of many malignancies. This approach increases patient survival but carries risks of irAEs. These complications need to be actively recognized by neurologists in an era with increasing use of these medications in the routine clinical oncology setting, and are likely to be more common than many of the recently described antibody-mediated illnesses.[8]

The patients we report showed a range of classical antibody-mediated conditions of the central and peripheral nervous system, and shared three intriguing features. Firstly, there was a stereotyped four-week lag from immune checkpoint-inhibitor administration to symptom onset, which has mechanistic implications discussed below. Secondly, and by contrast to a recent series in MG,[10] despite clinical presentations and investigation findings indistinguishable from traditional seropositive equivalents, none showed the common autoantibody profiles associated with their respective conditions. However, two patient sera showed novel autoantibody reactivities with disease-relevant preparations, including one on a live cell system, suggesting study of these patients may hasten the discovery of antigenic targets in conventional antibody-mediated diseases. Finally, all patients showed a very good recovery with symptomatic therapies or immunotherapies including corticosteroids, intravenous immunoglobulins and/or plasma exchange, without residual clinical deficits or atrophy. This consistent improvement, in addition to the novel observed autoantibody reactivities, suggest antibody-mediated effector neurological mechanisms rather than the more commonly-hypothesized checkpoint-inhibitor induced T cell-mediated conditions which affect the skin, gut and liver.[11,12] Indeed, the concept of an autoantibody-mediated condition is supported by the recent discovery of increased plasmablasts,[13] and a few organ-specific autoantibodies, post-checkpoint inhibitors.[14]

In addition to hypophysitis, neurological side-effects of the checkpoint inhibitors include very few or single cases of aseptic meningitis, GBS and myositis.[5,15] More recently, 0.1% of patients administered nivolumab were reported to develop MG. Furthermore, multiple sclerosis after CTLA-4 blockade by ipilimumab has been reported in one case where the clonally-expanded T-cell receptor sequences shared between the CSF and melanoma suggest pre-formed T-cell memory led to the CNS disease.[16] Similar close clonal relationships of T cells are recognised between tumor and cardiac tissue in post-checkpoint inhibitor myocarditis,[12] supporting the notion that many irAEs are T cell mediated.

Overall, the consistent four-week lag observed by us and two cases with autoimmune encephalitis after a similar interval from immune checkpoint inhibitor administration,[17] suggest insufficient time for generation of a *de novo* immune response to neurological antigens. More likely, the drug-induced disinhibition of circulating T cells led to the activation of preformed B cell reactivities to neural and neuromuscular proteins. This observation implies that autoantigen-specific T and B cells were circulating in an anergic or quiescent state, contained by Tregs: indeed, flow-cytometry from Patient 1 suggests the suppressive population were within the $CD4^{+}CD25^{+}CD62L^{+}$ Tregs bound by the monoclonal antibody pembrolizumab. Interestingly, the presence of pre-existing neural-antigen specific cells in those with or without disease may be consistent with the frequent detection of neurological autoantibodies, particularly of the IgM subclass, in healthy control subjects.[18] Similarly, screening of patients without neurological symptoms who have tumors and paraneoplastic autoantibodies,for example $GABA_B$-receptor antibodies and small cell lung carcinoma,[19] may identify those with a potentially increased rate of irAEs. As an alternative to a direct T cell mechanism of action, as PD-1 and CTLA-4 are also expressed on B cells and some myeloid cells, and PD-L1 on neurons and tumor cells, the checkpoint inhibitors may have additional direct actions on cells other than T cells.[2] These mechanisms should be investigated in future studies.

In summary, antibody-mediated neurological complications require increased clinical vigilance given the escalating use of immune checkpoint inhibitors. Further study of these patients may highlight immunological mechanisms operating in all antibody-mediated diseases, and have implications for the detection of anergic autoreactive T and B-cells in healthy controls.

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Figure 1. Radiological and immunological features of patient 1. A T2 weighted STIR sequence with extensive medullary cord edema and swelling extending from the medulla oblongata into the low thoracic spinal cord. B. The pons also shows T2 weighted bilateral circumscribed areas of high signal on axial imaging. C and D. After 4 months and corticosteroids, intravenous immunoglobulins and plasma exchange, both sagittal and axial images show almost complete resolution of swelling with no persistent gliosis or atrophy. E. Flow cytometry plots show $CD3^{+}CD4^{+}$ T cells gated on CD25 and CD127 to identify Tregs ($CD25^{+}CD127^{lo}$) in healthy control (HC) peripheral blood. F. Enumeration revealed a reduction of Tregs in the blood of patient 1. G. Tregs from patient 1 contained a subpopulation of $CD25^{++}CD62L^{++}$ cells, all of which stained positively for human IgG, representing bound humanized pembrolizumab (blue contours). H. This population was not bound by IgG in the healthy controls.

Figure 2. Novel autoantibody reactivities in patients with checkpoint inhibitors. A. Aquaporin-4 antibody positive (AQP4), healthy control (HC) and patient number 1 (Pt1) serum IgG binding to rodent sections of cerebellum (left column) and hippocampus (right column). Scale bar = 500 µm. Paraformaldehyde-fixed brain sections were incubated for one hour with patient serum (1:200 dilution in PBS / 0.1% Triton-X100 / 5% bovine serum albumin), washed in PBS / 0.1% Triton X100, and then incubated with anti-human HRP-conjugated secondary antibodies (1:750) for 45 minutes. Visualization with 3,3'-diaminobenzidine and hydrogen peroxide. B. Higher magnification showing HC and patient 1 (Pt1; C) IgG binding to cerebellar granule cells (G) more than molecular layer (M) or white matter (W). Scale bar = 100 µm. D. Serum IgM (1:100 diliution for 1 hour at 37°C) from the patient with Guillian-Barre syndrome bound live myelinating co-cultures (from human induced pluripotent stem cell derived sensory neurons and rat

primary Schwann cells). Subsequently, cultures were fixed in 1% paraformaldehyde and labelled with AlexaFluor-488 anti-human conjugated antibodies (green). The observed binding was to myelin blebs in particular. This was followed by permabilisation with ice cold methanol (30 min, on ice), and counter-labelling anti-neurofilament-heavy (1:10,000, labelled blue, NF200) and anti-myelin basic protein (1:500, labelled red, MBP) primary antibodies to visualise axonal processes and myelin internodes, respectively.

	Patient 1	Patient 2	Patient 3	Patient 4
Age	35	57	62	52
Sex	Male	Male	Female	Male
Tumour	Classical Hodgkin lymphoma	Melanoma	Lung adenocarcinoma	Melanoma
Checkpoint inhibitor(s)	Pembrolizumab	Nivolumab and ipilimumab	Pembrolizumab	Nivolumab and ipilimumab
Time from administration to symptoms	4 weeks	4 weeks	4 weeks	4 weeks
Clinical features	Tetraparesis, sensory level, loss of sphincters	Fatiguable ptosis and complex external ophthalmoplegia	Fatiguable ptosis and limb weakness	Sensory loss and reduced reflexes
Clinical diagnosis	Longitudinally extensive transverse myelitis	Myasthenia gravis	Myasthenia gravis	Guillain-Barre syndrome
Novel serum autoantibody?	Yes, IgG	No	No	Yes, IgM
Negative antibody results	AQP4, MOG, CRMP5, GFAP, amphiphysin	AChR (including clustered), MuSK and LRP4	AChR (including clustered), MuSK and LRP4	Gangliosides, CRMP5, GFAP Contactin-1, CASPR1, NF140/155/186
Nerve conduction / EMG studies	Not performed	Normal	Normal	Prolonged distal motor latencies, low conduction velocities and absent F waves.

Treatment for neurological features	Corticosteroids, intravenous immunoglobulins and plasma exchange	Corticosteroids	Pyridostigmine and corticosteroids	Intravenous immunoglobulins and corticosteroids
Oncological Outcome	Complete remission	Death, progression of metastases	Stable lung tumor, resolution of metastases	Reduction in tumor load
Neurological Outcome	Excellent, mild residual hypertonia	Complete	Complete	Complete
Follow up period	2.5 years	6 months	1 year	1 year

Table 1. Clinical and investigation features of patients with neurological complications after checkpoint-inhibitors. Patient 1 received adriamycin, bleomycin, vinblastine, dacarbazine, then ifosfamide, epirubicin and etoposide and finally brentuximab (CD30 targeting). No relapses of the tumor or neurology were noted. Aquaporin 4 (AQP4) antibodies were not detected on live and fixed cell based assays. CRMP5 and amphiphysin antibodies were detected by commercial line blot, and GFAP antibodies by fixed cell based assay. Other antibodies were tested by live cell based assays.[6,7] Patient 3 had no single fiber EMG performed, and EMG studies in both patients 2 and 3 were performed after treatment initiation. AChR = acetylcholine receptor, CASPR1 = contactin-associated protein 1, CRMP5 = collapsin response mediator protein 5, GFAP = glial fibrillary acidic protein, LRP4 = low density lipoprotein receptor-related protein 4, MOG = myelin oligodendrocyte glycoprotein, MuSK = muscle specific kinase, NF = neurofascin.

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Potential Conflicts of interest GC has received honoraria from Merck and BMS for advisory work and trial funding; SRI receives royalties as a co-applicant on a patent describing VGKC-complex antibodies, including LGI1, CASPR2 and contactin-2, licensed to Euroimmun Ltd.

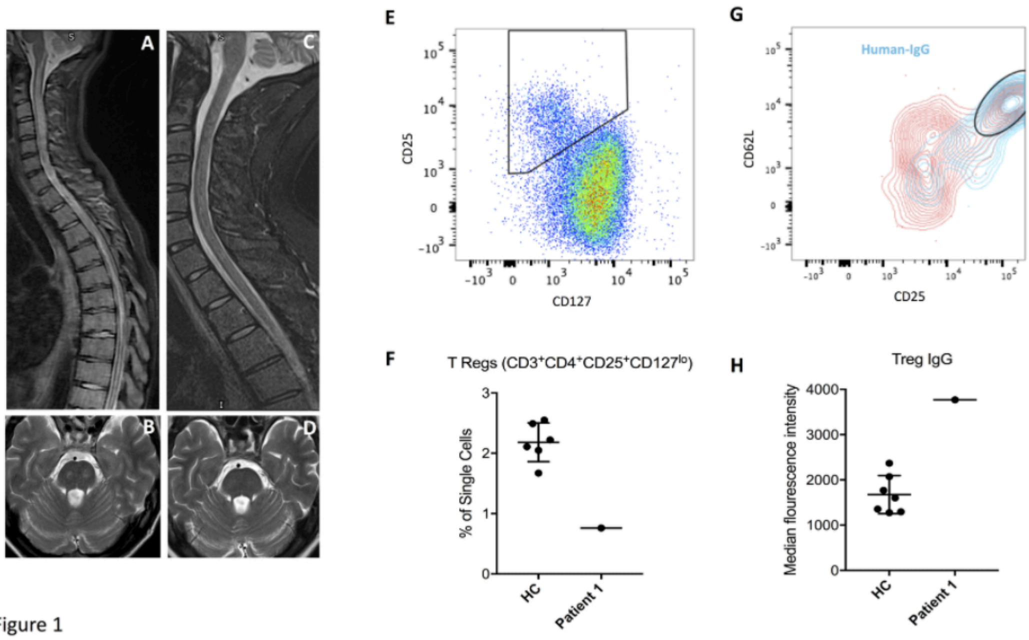
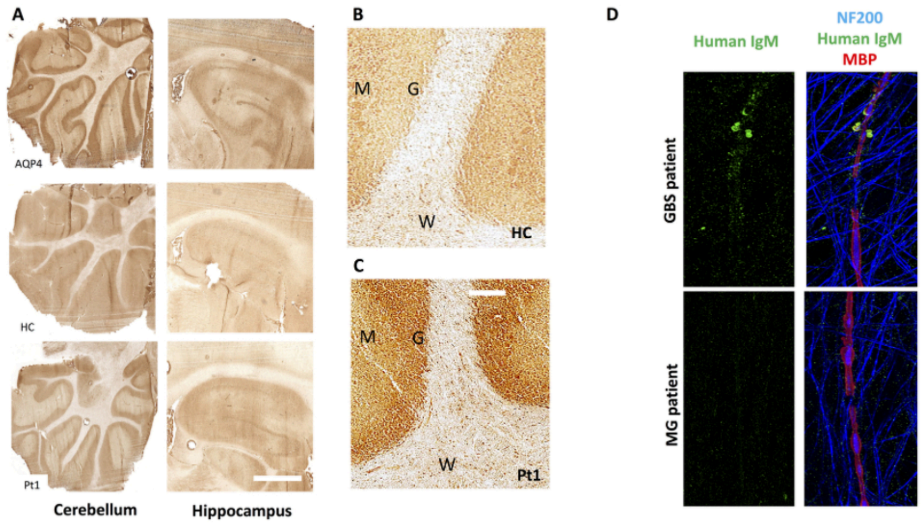


Figure 1



A
AQP4
HC
Pt1
Cerebellum
Hippocampus
B
M
G
W
HC
C
M
G
W
Pt1
D
Human IgM
NF200
Human IgM
MBP
GBS patient
MG patient

Figure 2