INTRODUCTION

Neuromyelitis optica spectrum disorders (NMOSDs) are mediated by antibodies directed against the extracellular domain of aquaporin-4 (AQP4). These antibodies form a key pillar in diagnostic criteria for NMOSD. Yet, the immunological mechanisms underlying the generation of AQP4 antibodies during disease initiation are incompletely understood, principally because this is an asymptomatic period. To date, AQP4 antibodies and symptomatic NMOSD are known to develop several years after myasthenia gravis, typically post-thymectomy, or in the context of bone marrow transplantation. These examples suggest the immunopathogenesis of symptomatic NMOSD typically requires many years to mature.

The duration and nature of the immune response maturation can provide insights into the cellular processes responsible for AQP4 antibody production, in particular the potential relevance of long-lived plasma cells versus germinat centre reactions. Hence, the fundamental immunopathogenesis may inform the rational selection of targeted immunotherapeutics.

Here, we describe a patient who developed post-transplant NMOSD, and capture the key period of acute clinicoradiological disease conversion with serial biological samples. The findings revealed herein provide several unique insights into the immunopathogenesis of NMOSD.

MATERIALS AND METHODS

Phenotype and patient samples. Clinical and radiology data collection was prospectively gathered, along with serial blood samples (both cells and serum), and archived for research purposes.

AQP4-antibodies. Live cell-based assays were performed, with minor modifications from published protocols. In brief, HEK293T cells were transfected with cDNA encoding full-length AQP4 and, while live, labelled with patient IgG or IgM which, after fixation, were detected with isotype-specific secondary antibodies (product numbers 709-585-098, Jackson labs, and A-21216, Thermofisher, respectively). Prior to AQP4-IgM detection, IgGs were fully depleted with protein G beads. All positive results were titrated to endpoint dilutions.

B cell populations. From liquid nitrogen archived whole blood, mass cytometry immunophenotyped several populations including B cells (details in online supplemental data). Naïve B cells were defined as CD19+CD20+CD27−.

RESULTS

Clinical features

A boy (between 1 and 2 years of age) with STAT3 gain-of-function mutation received a matched unrelated donor peripheral blood stem cell transplant to treat severe refractory multisystem autoimmune disease, including neonatal giant cell hepatitis and complete lipodystrophy.

After an unremarkable early post-transplant course, on day 49 he developed a fever and respiratory distress, with no infective cause identified (figure 1A). On day 61, oedema, rash and diarrhoea led to a diagnosis of graft-versus-host disease (GVHD), confirmed on upper gastrointestinal tract biopsy and treated with methylprednisolone (2 mg/kg) from day 68. Subsequently, on day 76, he developed severe vomiting, initially considered secondary to progressive GVHD. However, after 1 week he had slow pupillary reactions, left-sided weakness, a decreased level of consciousness and apnoea. MRI showed T2 hyperintense lesions predominantly affecting the pons, medulla, area postrema and cervical cord (figure 1B), with optic nerve sparing. Serum AQP4-IgG was detected with normal total immunoglobulin levels. He was diagnosed with NMOSD and treated aggressively with 30 mg/kg methylprednisolone, plasmapheresis and alemtuzumab (0.2 mg/kg×5 doses). On day 93, he developed labile blood pressure and
fixed-dilated pupils. Repeat MRI showed brainstem lesion extension plus new bithalamic involvement (figure 1B). The neurological disease was considered irreversible and respiratory support withdrawn on day 94.

**Laboratory findings**

Retrospective live cell-based assays showed the de novo appearance of serum AQP4 antibodies (1:80 endpoint dilution) on day 67, with levels which rose to 1:160 by day 76 (figure 1A). After confirmed depletion of IgG, these two samples additionally showed AQP4-IgM reactivities (1:40 and 1:80 endpoint dilutions, respectively). No other samples showed AQP4-IgM or AQP4-IgG. Mass cytometry analysis revealed that 70% of the B lymphocyte population, between days 25 and 38, rising from 0.8% to 72% of all leucocytes (figure 1A–C). This time course represents a highly accelerated reconstitution of the naïve B cell compartment, which is usually delayed until >6 months post-transplant.

Genotyping on day 83 (a comparison of donor and recipient DNA using PowerPlex 16 HS system) revealed that 70% of CD19 + cells were donor derived (30% were from the recipient); whereas none of the residual CD3 + T cells and only 21% of myeloid cells were donor derived.

**DISCUSSION**

This tragic case provides a unique opportunity to observe a de novo human autoimmune reaction against AQP4. Below, we synthesise longitudinal clinical, cellular and serological observations from this distinctive case to hypothesise mechanisms of AQP4 antibody synthesis, with both clinical and therapeutic relevance.

The temporal dynamics of this human autoimmunisation identified the generation of AQP4 antibodies over just a few weeks, early after stem cell transplantation and more acutely than documented in two different clinical scenarios. An unusually sharp ~100-fold rise in naïve B cells occurred prior to generation of AQP4 antibodies. This time course may reflect the exit of donor antigen-inexperienced B cells from the bone marrow (70% of the B cells were donor derived) and their subsequent maturation towards precursors of the serum AQP4 antibodies.

Around 1 month later, both de novo serum AQP4-IgG and IgMs were observed and temporally coincided with the development of symptomatic NMOSD. The concurrent AQP4-IgG and IgMs suggest an acute immunisation in this patient (akin to that observed in many infections), and support a germinal centre-based generation of AQP4 antibodies. This germinal centre activity may be fuelled by the reconstituting naïve B cells which, in patients with NMOSD, have been observed to both carry AQP4 reactivities and show deranged regulatory properties. Hence, prevention of naïve B cell reconstitution, for example, with anti-CD19 and/or anti-CD20 drugs, may offer an important therapeutic target which represents a potential precursor to relapses in NMOSD. In further support of this mechanism, a few weeks is likely too short a duration to generate a significant population of human long-lived plasma cells. Yet, it remains possible that the AQP4-IgG generation resulted from incomplete depletion of plasma cells prior to transplantation.

STAT3 is a pleotropic transcription factor expressed by the NMOSD-associated Th17 T cell subset, which also drives the differentiation of T follicular cells and inhibits the generation of T regulatory cells. Therefore, it may be that disordered STAT3 signalling, particularly from the recipient’s residual T cells, could be implicated in the pathogenesis of their NMOSD. In summary, by detailing a case with an early, severe neurological complication after stem cell transplantation, we provide an opportunity to observe in vivo the development of AQP4 antibodies. Our data support a role for naïve B cells and germinal centres in the initiating pathogenesis of NMOSD. This conclusion has important implications for understanding disease pathogenesis and selecting optimal therapeutics.

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**Figure 1** Time course of cellular immune reconstitution: naïve B cell repopulation, AQP4 antibody generation and imaging changes following stem cell transplant. (A) Time course of peripheral blood naïve B cell counts (red, right y-axis), and serum AQP4-IgG (blue solid line, left y-axis) and AQP4-IgM (blue dashed line, left y-axis) endpoint dilutions. The first sample obtained was on day 67, with levels which rose to 1:160 by day 76 (figure 1A). After confirmed depletion of IgG, these two samples additionally showed AQP4-IgM reactivities (1:40 and 1:80 endpoint dilutions, respectively). No other samples showed AQP4-IgM or AQP4-IgG. Mass cytometry analysis revealed that 70% of the B lymphocyte population, between days 25 and 38, rising from 0.8% to 72% of all leucocytes (figure 1A–C). This time course represents a highly accelerated reconstitution of the naïve B cell compartment, which is usually delayed until >6 months post-transplant.
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Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jnnp-2022-328982).

Open access Article

To cite McNaughton P, Payne R, Michael S, et al. J Neurol Neurosurg Psychiatry 2022;93:1234–1236. Received 4 February 2022 Accepted 20 April 2022 Published Online First 23 May 2022 J Neurol Neurosurg Psychiatry 2022;93:1234–1236. doi:10.1136/jnnp-2022-328982

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Supplementary data

Mass cytometry.
Briefly, white blood cells (WBCs) were prepared from lysed whole blood and live/dead stained using Cell-ID cisplatin (5μM, Fluidigm, 201064). After washing, WBCs were surface stained, at room temperature, with primary (30 minutes) and secondary (60 minutes) antibodies, washed and fixed using 1.6% formaldehyde for 1 hour. Fixed cells were washed and cryopreserved in freezing media (FBS + 10% DMSO). Thereafter, thawed samples were washed and permeabilized using 0.1% Triton X-100, washed and stained intracellularly overnight at 4°C. Finally, cells were retrieved, washed and intercalated for 1 hour (Ir-125μM, 1:1000, Fluidigm, 201192A) in Maxpar fix/perm buffer (Fluidigm, 201067) and acquired immediately on a Helios mass cytometer platform (Fluidigm). Normalized and bead excluded FCS files were analysing within a R pipeline, populations were clustered and visualised using FlowSOM and ConsensusCentrePlus wrapped within CATALYST package.

B populations were determined using antibodies against CD19, CD20, CD27 and IgD.