The immunopathogenesis of paraneoplastic neurological syndromes

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ABSTRACT
Paraneoplastic neurological syndromes are rare non-metastatic complications of cancer that have an immune-mediated aetiology. The central and peripheral nervous systems are considered to be immune-privileged sites, since the presence of the 'blood–brain/nerve barrier' means that antigens sequestered within the nervous system do not normally induce an immune response. Aberrant expression of a neuronal antigen by a tumour arising outside this barrier can lead to the breakdown of immune tolerance to the nervous system. However, in many cases the immune mechanisms that result in neurological dysfunction remain poorly defined. Furthermore, aberrant expression of neuronal antigens can be detected in many tumours that are not complicated by non-metastatic neurological syndromes. This review article examines current concepts in the immunopathogenesis of paraneoplastic neurological syndromes.

INTRODUCTION AND HISTORICAL PERSPECTIVE
Paraneoplastic neurological syndromes (PNSs) are a heterogeneous group of disorders that arise as non-metastatic manifestations of malignancy. The first description of a PNS recorded in the English scientific literature was reported in 1948 by Denny-Brown [1]. This report contained the clinical details of two patients with sensory symptoms in whom an associated bronchogenic carcinoma was found at post mortem. Both cases had marked sensory ataxia with one patient so severely affected that he was "quite unaware of the position of his tongue". Post-mortem examination revealed no evidence of tumour metastasis to the nervous system and noted that the dorsal root ganglia of both patients were profoundly abnormal with hardly a single dorsal root cell (cell body of the sensory neurone) remaining. Despite the observation that the dorsal root ganglia contained perivascular accumulations of lymphocytes it was concluded that in both patients "degenerative nervous and muscular affection is entirely consistent with that resulting from metabolic disorder". Since the pathological appearances of the dorsal root ganglia resembled those seen in sensory ganglionic degeneration in swine with pantothenic acid deficiency, it was proposed that the loss of dorsal root cells arose because the tumour in each case produced a "by product", such as thiopanic acid or phenyl pantothenone, that interfered with pantothenic acid metabolism.

By the 1950s it was realised that cancer-associated non-metastatic neurological signs and symptoms could extend beyond the sensory pathways [2,3] and that the perivascular aggregation of lymphocytes could involve all parts of the neuraxis [4] (Figure 1). In 1964 Wilkinson reported that the serum of five cases of cancer-associated sensory neuropathy contained complement-fixing antibodies that were organ-specific for central nervous tissue and dorsal root ganglia [5]. The following year, Wilkinson, in association with Zeromski, reported that
anti-brain antibodies from the same five patients could also be detected using indirect immunofluorescence against guinea-pig brain [6]. The detection of organ-specific anti-neuronal antibodies within the serum of patients with non-metastatic neurological complications of malignancy, coupled to the post mortem findings of perivascular and interstitial lymphocytic infiltrates in the brain and dorsal root ganglia, led Wilkinson to suggest that “… the tumour in a patient with sensory neuropathy may contain antigenic determinants not present in other tumours, and that these determinants are shared by some constituent of the central nervous system (CNS). An immune reaction against such tumour determinants might then incidentally cause damage to the CNS” [5]. Subsequent research has confirmed that aberrant expression of neuronal antigen by tumour may be associated with an immune response capable of breaching the blood–brain barrier, which normally results in the central and peripheral nervous systems being considered as immune privileged sites.

The clinical usefulness of detecting anti-neuronal paraneoplastic antibodies is now well established. In up to two-thirds of cases of PNS the neurological disorder arises prior to the diagnosis of the associated malignancy [7–9] and detection of a paraneoplastic anti-neuronal antibody in the serum of an individual with an apparently ‘idiopathic’ neurological syndrome defines the paraneoplastic aetiology of the disorder. Furthermore, determining antibody specificity can indicate likely sites of malignancy (see Table 1).

The majority of anti-neuronal antibodies currently known to be associated with PNS can be detected using indirect immunofluorescence (or immunohistochemistry). However, anti-voltage-gated calcium channel antibodies (anti-VGCCs) can only be reliably detected using an immunoprecipitation assay [10] and in many cases of PNS no anti-neuronal antibodies are detected using currently available methodology. Prior to identification of their antigenic targets, those anti-neuronal antibodies detected by indirect immunofluorescence were classified using a descriptive nomenclature that

### Table 1: Some paraneoplastic anti-neuronal antibodies, their antigenic targets and tumour associations

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Also termed</th>
<th>Antigen</th>
<th>Neurological syndrome</th>
<th>Associated tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-VGCC</td>
<td>P-type VGCC</td>
<td>LEMS</td>
<td>Cerebellar degeneration</td>
<td>SCLC (&gt; 80%)</td>
</tr>
<tr>
<td>Anti-Hu</td>
<td>ANNA-1</td>
<td>HuD</td>
<td>PEM/SSN</td>
<td>SCLC (75–80%) Neuroblastoma</td>
</tr>
<tr>
<td></td>
<td>Type IIa</td>
<td>Neuronal nuclear</td>
<td>Cerebellar degeneration</td>
<td></td>
</tr>
<tr>
<td>Anti-Yo</td>
<td>APCA-1</td>
<td>cdr 62, 34</td>
<td>Cerebellar degeneration</td>
<td>Ovary-gynaecological</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Purkinje cytoplasmic</td>
<td></td>
<td>Breast</td>
</tr>
<tr>
<td>Anti-Ri</td>
<td>ANNA-2</td>
<td>Nova 1, 2</td>
<td>Brainstem-cerebellar</td>
<td>Breast (50%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neuronal nuclear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Ma1</td>
<td>Ma1, 2, 3</td>
<td>Brainstem-cerebellar</td>
<td></td>
<td>Various</td>
</tr>
<tr>
<td></td>
<td>Anti-Ta</td>
<td>Ma2</td>
<td>Brainstem-cerebellar/limbic</td>
<td>Testis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neuronal nucleolar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-Tr</td>
<td></td>
<td>Purkinje cytoplasmic</td>
<td>Cerebellar</td>
<td>Hodgkins</td>
</tr>
<tr>
<td>Anti-GluR</td>
<td></td>
<td>Glutamate receptor</td>
<td>Cerebellar</td>
<td>Hodgkins</td>
</tr>
<tr>
<td>Anti-retinal</td>
<td></td>
<td>Recoverin</td>
<td>Retinopathy</td>
<td>SCLC</td>
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<tr>
<td></td>
<td></td>
<td>Photoreceptors</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Ganglion cells</td>
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<td></td>
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<tr>
<td>Anti-amphiphysin</td>
<td></td>
<td>Amphiphysin</td>
<td>Stiff-man syndrome</td>
<td>SCLC</td>
</tr>
</tbody>
</table>
referred to the staining patterns determined by target antigen distribution in the central and peripheral nervous system [11]. For example, APCA refers to an antibody that reacts with a Purkinje cell cytoplasmic antigen (Anti-Purkinje cell Cytoplasmic Antibodies). However, subsequent characterization of antigenic targets has led to the majority of investigators defining antibodies according to the name given to the antigenic target [11]. Although the additional usefulness of Western blot to determine paraneoplastic antibody specificity remains a hotly debated issue [11,12], in practice the majority of investigators recommend that the specificity of any antineuronal antibody detected by indirect immunofluorescence (or immunohistochemistry) is confirmed by Western blot against recombinant antigen. The rationale for utilizing Western blot against recombinant antigen relies on the fact that even experts can have difficulty distinguishing between antibodies of different antigenic specificity that react with target antigens of similar distribution (e.g. APCA-1 [11], PCA-2 [13] and anti-Tr [14] antibodies). Since clinical decisions are based on the correct identification of paraneoplastic anti-neuronal antibodies (see above), Western blot against recombinant antigen prevents reporting of false positive results detected by immunofluorescence methods in isolation. Furthermore, for the purposes of this article, which examines the pathogenesis of PNS, definitive knowledge of the antigenic targets is essential and paraneoplastic anti-neuronal antibodies that are detected by indirect immunofluorescence will be classified using the nomenclature proposed by Dalmau and Posner [12].

In this review we will describe some of the clinical syndromes associated with paraneoplastic antibodies reactive with well-characterized neuronal antigens and discuss the immunopathogenesis of neurological dysfunction. It should be noted that this is not a comprehensive review of all PNS and it must also be stressed that anti-neuronal antibodies are not always detected in patients with non-metastatic neurological complications of cancer.

The Lambert–Eaton myasthenic syndrome (LEMS)
LEMS is the commonest PNS, affecting 1–2% of patients with a diagnosis of small-cell lung cancer (SCLC) [15]. However, one-third of cases of LEMS occur in the absence of a detectable malignancy at diagnosis, and this sub-group can be considered to have a different autoimmune aetiology since cancer is not detected even with long-term follow up [16]. LEMS is characterized clinically by proximal lower limb weakness, potentiation of depressed tendon reflexes following sustained (10–15 s) maximum voluntary contraction, autonomic features (particularly dry mouth), and ptosis [7]. The clinical features predominantly reflect a defect in neuromuscular [7] and autonomic transmission and single fibre electromyography (EMG) studies show increased jitter, which is the characteristic electrophysiological finding in disorders of neuromuscular transmission. Nerve conduction studies undertaken to diagnose LEMS show small compound muscle action potentials and repetitive nerve stimulation at 3 Hz results in a decrement of the compound muscle action potential size. In contrast, 20 Hz repetitive nerve stimulation or 10–15 s of maximum voluntary contraction produces an increase in the size of the compound muscle action potential by up to 4774% [7]. Neurotransmitter release from pre-synaptic neurones at the neuromuscular junction and post-ganglionic sympathetic and parasympathetic neurones is mediated via depolarization of P-type VGCCs [17] and antibodies reactive with P-type VGCCs can be detected in the serum of over 85% of patients with clinically and electrophysiologically definite LEMS [10].

In addition to neuromuscular and autonomic symptoms, patients with LEMS may develop manifestations of CNS dysfunction. Since both LEMS and anti-Hu antibody associated PEM/SSN (where PEM/SSN stands for paraneoplastic encephalomyelitis and subacute sensory neuropathy; see below) usually arise as a complication of SCLC, the co-existence of these disorders in some SCLC patients may account for the associated CNS deficits. However, a subgroup of patients with LEMS and cerebellar dysfunction do not have anti-Hu antibodies and since P-type VGCCs, the target of the autoimmune process in LEMS, are also expressed by cerebellar Purkinje cells it is possible that cerebellar dysfunction could be mediated by anti-VGCC antibodies [18].

Figure 2 Anti-Hu antibodies detected using indirect immunofluorescence
A FITC-conjugated species-specific anti-human antibody detects anti-Hu antibodies reactive with myenteric plexus neurones in a monkey stomach section. The strongest staining is seen in the nucleus, but less intense staining is seen in the surrounding cytoplasm. The absence of nuclear staining is seen best in the neurone in the bottom right hand corner, appearing as a dark area within the otherwise bright fluorescent nuclear staining. (Reproduced from Advanced Atlas of Autoantibody Patterns with permission, courtesy of Professor AR Bradwell, Department of Immunology, University of Birmingham [89].)
**PEM/SSN and anti-Hu antibodies**

Anti-Hu antibodies can be readily detected using indirect immunofluorescence (Figure 2) and are found in the serum of patients with a wide spectrum of neurological syndromes, including SSN, motor neurone dysfunction, autonomic neuropathy, brainstem encephalitis, limbic encephalitis and cerebellar dysfunction [8]. The term PEM/SSN has been coined to encompass these diverse clinical syndromes since more than one clinical syndrome can affect any individual patient. The diversity of neurological dysfunction found in patients with PEM/SSN reflects the fact that Hu antigens, the target of autoimmune dysfunction, are expressed in all central and peripheral neurones [19]. Deposits of anti-Hu IgG can be demonstrated in the nervous system of patients with PEM/SSN and there is a limited correlation between the principal clinical symptoms, regions of major tissue injury, and quantitative anti-Hu IgG distribution [20].

However, the majority of the perivascular and interstitial lymphocytic infiltrate in the CNS and dorsal root ganglia is made up of CD8+ T lymphocytes [21–23] suggesting that cellular immune mechanisms may also contribute to the neurological dysfunction.

The Hu antigens are four proteins, HuD, HuC, HuR and Hel-N1, that share significant sequence homology and make up the Elav family of RNA binding proteins. HuD, HuC and Hel-N1 are expressed in terminally differentiated neurones and HuR is expressed in all proliferating cells [24]. These antigens bind to AU-rich elements in the 3’-untranslated regions of mRNAs, some of which encode proteins involved in regulation of cell proliferation [24]. Whilst their precise function is unknown it is proposed that HuC, HuD and Hel-N1 differentially upregulate mRNAs encoding protein products necessary for neuronal development and function [24].

Approximately 75% of cases PEM/SSN associated with anti-Hu antibodies arise as a non-metastatic complication of SCLCs [8,25]. SCLCs are derived from neuro-endocrine Kulchitsky cells and HuC, HuD and Hel-N1 are expressed by all SCLCs, not just those associated with PEM/SSN [19]. Hu antigens are also expressed by 50–78% of neuroblastomas [19,26], another tumour derived from neuro-endocrine cells that is known to associate with anti-Hu antibodies. Tumours of non-neuro-endocrine origin associated with PEM/SSN and anti-Hu antibodies aberrantly express Hu antigens. A recent study that examined 14 extra-thoracic non-SCLC tumours associated with PEM/SSN–anti-Hu antibodies demonstrated aberrant Hu antigen expression in all 14 tumours using biotinylated anti-Hu IgG [25].

**Paraneoplastic cerebellar degeneration (PCD) and anti-Yo antibodies**

PCD results in a characteristic clinical syndrome, but it is apparent that the immunopathogenesis of PCD is heterogeneous. It has already been noted that PCD can arise in patients with anti-Hu and anti-VGCC antibodies, and reference to Table 1 reveals that no less than eight different antibodies reactive with Purkinje cell antigens are associated with PCD. In patients presenting with a paraneoplastic syndrome manifesting predominantly as cerebellar dysfunction the most frequently detected anti-Purkinje cell antibody reacts with an antigen termed Yo or cdr2 (cerebellar degeneration related 2) [27,28]. Anti-Yo antibodies are usually found in female patients with cerebellar dysfunction [29], with only three reported cases of anti-Yo antibody detection in similarly affected males [30–32]. Gynaecological and breast malignancies are the usual tumour associations in females [29], whereas all the three male cases had adenocarcinomas (oesophageal, parotid, unknown primary) [32].

Anti-Yo antibodies react against at least two cerebellar degeneration related antigens, a major 62 kDa species and a minor 34 kDa species [27]. The mRNA for the 62 kDa Yo protein is found in cells throughout the body but a post-translatory mechanism normally confines protein expression to Purkinje cells and cells within the testis (another immune-privileged site) [28]. However, the Yo antigen is frequently expressed by ovarian and breast cancers [33], and Furneaux et al. [34] demonstrated Yo expression in ten breast/gynaecological tumours associated with cerebellar degeneration and anti-Yo antibodies. The 62 kDa antigen was aberrantly expressed in all ten cases and the cdr1 (34 kDa) species was additionally expressed by one of these tumours. A smaller study confirmed that the 62 kDa was the only antigen expressed by three ovarian tumours [28], suggesting that aberrant expression of the 62 kDa Yo antigen by the tumour appears to be critical in the breaking of immune tolerance.

The major post mortem finding in patients with cerebellar degeneration associated with anti-Yo antibodies is the virtual complete loss of cerebellar Purkinje cells (Figure 3) suggesting that immune-mediated mechanisms are targeted towards the Yo antigen. However, the number of published pathological studies on patients with anti-Yo antibody associated PCD are small and offer little insight into the nature of the immune processes that might result in PCD. Three cases reported by Peterson et al. [29] demonstrated scattered T lymphocytes in the leptomeninges, with no inflammatory infiltrate in the cerebellar cortex. Verschuuren et al. [35] reported post mortem findings in two patients and whilst identifying inflammatory infiltrates in the medulla and pons in one patient found no evidence of inflammation in the cerebellum of either patient. This study concluded that the loss of Purkinje cells represented a final ‘burn out’ stage of a cell-mediated immune process. This hypothesis is supported by the report of a CD8+ inflammatory infiltrate in the cerebellar cortex of a patient who died of myocardial infarction 4 months after the onset of cerebellar symptoms [36].
Figure 3 Anti-Yo antibodies and Purkinje cell loss in PCD

Top panel: anti-Yo antibodies are detected using indirect immunofluorescence and react with a cerebellar Purkinje cell cytoplasmic antigen producing a granular staining pattern. (Reproduced from Advanced Atlas of Autoantibody Patterns with permission, courtesy of Professor AR Bradwell, Department of Immunology, University of Birmingham [89].) Middle panel: post-mortem cerebellum section (H&E; haematoxylin and eosin) demonstrating that the cerebellar cortex is composed of the granular layer (G), the Purkinje cell layer (P) and the molecular layer (M). Bottom panel: post-mortem cerebellum section (H&E) from a patient with anti-Yo antibodies and paraneoplastic cerebellar degeneration. There is complete absence of Purkinje cells indicating that the Purkinje cells are the targets of autoimmune dysfunction.

The major Yo antigen (cdr2) has been identified as a 62 kDa protein with leucine-zipper and zinc-finger motifs [27]. Although leucine-zipper motifs are known to bind to DNA, electron microscopy studies localized this antigen to free and membrane-bound ribosomes [37]. The precise function of the Yo antigen is unknown, but it is known that Yo interacts with a helix-leucine motif of c-myc causing its redistribution within the cytoplasm [38].

Other paraneoplastic disorders of the CNS

A number of other paraneoplastic disorders of the CNS are associated with the presence of paraneoplastic antineuronal antibodies that react with well-characterized antigens.

Anti-Ri antibodies were first identified in a patient with paraneoplastic opsoclonus arising as a complication of breast cancer [39]. Despite the fact that this striking oculomotor disorder characterized by involuntary saccadic eye movements has virtually become synonymous with anti-Ri antibodies, opsoclonus has only been seen in half of the reported cases [40]. However, those patients that do not develop opsoclonus, almost always have another eye movement disorder and clinical examination indicates that the brainstem structures are the major target of autoimmunity. Nevertheless, probing cDNA libraries with anti-Ri sera has defined two antigens, Nova-1 and Nova-2, that are widely expressed within the CNS [41] and post-mortem studies on three patients with anti-Ri antibodies show evidence of widespread perivascular and interstitial lymphocytic infiltrate in the CNS [42–44]. Although patients with anti-Ri antibodies may have evidence of cognitive dysfunction, myelopathy or basal ganglia abnormalities it is unclear as to why the brainstem structures appear to be most susceptible to the inflammatory processes associated with anti-Ri antibodies.

The Ma proteins (Ma1, Ma2 and Ma3) are a novel family of brain-testis-cancer proteins that have recently been identified as the target antigens of antibodies in individuals with paraneoplastic hypothalamic-limbic encephalitis and/or brainstem encephalitis and cerebellar degeneration [45–47]. As is the case with the Yo antigen, the Ma1 antigen is also expressed in the testis [46], otherwise Ma antigen expression is restricted to neurones and the highest levels of expression are seen in the limbic structures, tegmental nuclei and the cerebellar dentate nucleus. Aberrant expression of the Ma antigens can also be demonstrated in the tumours of affected individuals [45–47].

In contrast to other paraneoplastic disorders magnetic resonance imaging is often abnormal in this patient group. Signal changes on T2-weighted images, consistent with an underlying inflammatory process, can be seen in the medial temporal lobes, thalamus/basal ganglia, hypothalamus and brainstem [47–49] (Figure 4), but these changes may not be apparent until 8 weeks after presentation with clinical symptoms and signs [47]. Nevertheless, it should be noted that the appearances seen on T2-weighted imaging are not specific for an inflammatory process and enhancement of these lesions can occur following administration of gadolinium, thereby mimicking the appearances of metastatic tumour. However, biopsies of these lesions and post-mortem studies have demonstrated that these lesions contain
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Figure 4 T2-weighted magnetic resonance image of a patient with paraneoplastic brainstem encephalitis and anti-Ma2 antibodies showing signal change within the brainstem (arrow)

At post mortem there was perivascular lymphocytic cuffing and lymphocytic infiltration of the brainstem [48] [Neurol. Neurosurg. Psychiatry (2001) 70, 222–225, with permission from the BMJ Publishing Group].

perivascular and interstitial lymphocytic infiltrates that are characteristic of paraneoplastic neurological disorders [47,48].

Published data on 29 patients with immune responses to Ma proteins suggests two patterns of serum reactivity. Ma1, Ma2 and Ma3 share significant sequence homology and eleven patients had antibodies that react with Ma2, Ma1 and/or Ma3. A broad spectrum of tumour types are associated with this pattern of antibody reactivity [46,47].

Eighteen individuals are reported in whom antibody reactivity was limited to Ma2 (previously referred to as anti-Ta antibodies). Fifteen of these patients were male, of which 14 had an associated germ-cell tumour of the testis. This group of patients is of interest since complete response of the testicular cancer to treatment is often associated with a dramatic improvement in the neurological symptoms [47]. This is in contrast to other paraneoplastic disorders of the CNS, which rarely improve following tumour treatment or immunotherapy.

A number of studies have reported cerebellar ataxia arising as a non-metastatic complication of Hodgkin’s disease [50–52]. Whilst it has not been possible to characterize the target antigen of anti-Tr antibodies [14], that react with a Purkinje cell cytoplasmic antigen and are found in some of this patient group, antibodies reactive with the extra-cellular domain of the metabotropic glutamate receptor (GluR), mGluR1, have recently been described in two patients who developed cerebellar ataxia after achieving a remission from Hodgkin’s disease [53].

Immunopathogenesis of PNS

The preceding discussion on the clinical presentations and antigenic targets of immune dysfunction in patients with PNS highlights two important facts. Firstly, PNS are rare complications of malignancy and secondly, PNS arise because aberrant expression of a neuronal antigen by a tumour results in a breakdown of immune tolerance resulting in immune-mediated neuronal dysfunction. Despite being uncommon disorders, PNS continue to be extensively researched since there is a general belief that elucidating the immunopathogenesis of PNS will lead to an understanding of ways in which the immune system can be manipulated to provide therapeutic tumour immunity. The following discussion examines three areas of potential interest to neurologists, who manage PNS, and tumour immunologists interested in cancer immunotherapy.

What are the mechanisms of immune-mediated neuronal dysfunction?

Over 30 years ago identification of anti-neuronal antibodies in the serum of patients with non-metastatic complications of malignancy led to the advancement of the hypothesis that these disorders are immune-mediated conditions. Since patients with PNS often have evidence of high titres of anti-neuronal antibodies within the cerebrospinal fluid (CSF), indicative of intra-thecal antibody synthesis [54,55] subsequent investigation has examined possible mechanisms by which neurological dysfunction could be mediated by anti-neuronal antibodies.

Several criteria have to be satisfied before a disorder can be considered to be antibody mediated.

1. Antibody reactivity with the antigenic target results in a clinical phenotype compatible with antigen loss and/or loss of neurones expressing the antigen.
2. Passive transfer of the immunoglobulin fraction from an affected patient into an experimental animal reproduces the clinical phenotype in the animal.
3. Immunization of an experimental animal with purified antigen leads to development of an antibody response and this antibody response results in the clinical phenotype seen in patients with the same antibody reactivity.
4. Immunotherapy that leads to a reduction in titres of antibody is associated with clinical improvement or stabilization of symptoms.

Antibodies reactive with anti-VGCC and the anti-GluR have satisfied certain of the aforementioned criteria, thus they can be considered to be pathogenic antibodies.

LEMS is the most well established example of a PNS that can be considered to be an antibody-mediated disorder. The presence of a humoral factor that could account for the clinical manifestation of LEMS was
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Figure 5 Schematic representation of the immuno-pathogenesis of LEMS

When the nerve impulse reaches the nerve terminal depolarization of membrane VGCCs leads to transient opening and an influx of calcium results in quantal release of acetylcholine. In LEMS antibodies are directed against the pre-synaptic P-type VGCCs. Cross-linking of P-type VGCCs results in down-regulation leading to impaired quantal release of acetylcholine and impairment of neuromuscular transmission. Passive transfer of LEMS-IgG and LEMS-IgG (Fab), fragments into experimental animals can reproduce the electrophysiological and morphological changes seen in LEMS patients. Reproducing disease using (Fab)2 fragments demonstrates that Fc-mediated effector functions are not involved in the pathogenesis of LEMS. The importance of VGCC cross-linkage is confirmed by the failure of passive transfer of Fab fragments to reproduce disease in experimental animals.

suggested by the observation that clinical and electrophysiological parameters improved following plasma exchange [56]. It is now know that in over 85% of cases of LEMS patient serum contains increased levels of antibodies that react with the a1a subunit of the P-type VGCC [10]. Under normal conditions depolarization of the P-type VGCC at the pre-synaptic junction leads to an influx of calcium, which results in quantal release of acetylcholine and subsequent muscular contraction. A direct causal effect of anti-VGCCs in the pathogenesis of LEMS was demonstrated by electrophysiological examination of the mouse phrenic nerve-diaphragm preparation after passive transfer of IgG purified from LEMS patient sera into mice. The findings of reduced quantal release of acetylcholine and facilitation of end-plate potential amplitudes following high frequency phrenic nerve stimulation in animals that had been treated with LEMS patient IgG [57], reflect the electrophysiological characteristics identified by EMG studies of LEMS patients (see above). Furthermore, passive transfer of LEMS patient IgG to mice results in depletion of active zone particles thought to represent VGCCs [58]. Additional morphological data suggested that anti-P type VGCCs exert their effect by cross-linking active zone particles resulting in down-regulation, rather than via Fc-mediated effector mechanisms. This hypothesis was confirmed by demonstrating that divalent LEMS IgG and F(ab')2 could deplete active particle zones, whereas this effect was not observed with monovalent Fab [59] (Figure 5).

LEMS often co-exists with PCD and it has been suggested that the cerebellar dysfunction is also an antibody-mediated phenomenon. Circumstantial evidence for this hypothesis is that in addition to being expressed at the neuromuscular junction, P-type VGCCs are also expressed by cerebellar Purkinje cells. Furthermore, mutations in the CALC1N gene encoding the a1a subunit, the antigenic target of anti-VGCCs in LEMS, results in three neurological conditions in which cerebellar ataxia is a prominent manifestation: episodic ataxia type 2, familial hemiplegic migraine and spinocerebellar ataxia type 6 [60,61]. Although there is one report of raised anti-VGCC antibodies in the CSF of a patient with LEMS and PCD [62], as yet there is little evidence to confirm that PCD associated with LEMS is mediated by anti-VGCC antibodies.

Evidence that PCD can be a manifestation of an autoantibody-mediated process was recently established by studying of two patients who developed cerebellar ataxia after achieving a remission from Hodgkin’s disease [53]. High titres of antibodies reactive with the N-terminal extracellular domain of mGluR1 were identified in the serum and CSF of both patients and since mice that lack the target mGluR1 antigen have ataxia and intention tremor [63], this suggested that anti-GluR1 antibodies could have played a pathogenic role in the development of cerebellar ataxia. Functional effects of the anti-GluR1 antibody were demonstrated by comparing treatment of mGluR1 expressing Chinese hamster ovary cells with purified IgG from the patients and normal control subjects. IgG from patients, but not controls, inhibited glutamate-stimulated formation of inositol phosphates. Furthermore, both patients had evidence of intrathecal synthesis of anti-GluR1 antibodies and in-
jection of purified patient anti-GluR1 antibodies into the subarachnoid space of normal mice resulted in the development of ataxia [53].

Whereas anti-VGCC and anti-GluR antibodies both react against membrane target antigens, the target antigens of the other antibodies that are listed in Table 1 are intracellular proteins. To date there is no evidence supporting a pathogenic role for a paraneoplastic antibody that reacts with an intracellular antigen. Although both anti-Hu IgG and anti-Yo IgG can penetrate viable Hu [64] and Yo [65] antigen-expressing cells, PEM/SSN and anti-Yo associated PCD do not appear to be antibody mediated disorders. Both antibodies fail to meet three out of the four (2, 3 and 4) of the aforementioned criteria that must be met prior to an antibody being considered to have pathogenic activity.

Attempts to develop animal models of anti-Hu associated PEM/SSN and anti-Yo associated PCD have been unsuccessful. Passive transfer of anti-Hu containing IgG into experimental animals fails to reproduce neurological disease [66]. Two studies have administered intraventricular injections of IgG from patients with high titres of anti-Yo antibodies into experimental animals [67,68] and a further study injected animals with induced blood–brain barrier disruption intraperitoneally [65]. All three studies noted Purkinje cell uptake of anti-Yo antibodies, without clinical evidence of disease. Furthermore, no Purkinje cell loss could be induced by intraventricular injection of complement or lipopolysaccharide-activated human macrophages or rat mononuclear cells along with anti-Yo IgG [69]. Immunization of experimental animals with HuD [70] and Yo [70,71] produced high-titre antibody responses to both antigens, but no neurological disease was observed in either case. Additional studies in the latter case also failed to produce neurological disease when spleen cells from Yo-immunized mice were injected intracerebrally into naïve mice. In vitro studies have also been undertaken with anti-Hu antibodies and whilst one study showed anti-Hu containing IgG produced specific lysis of rat cerebellar granule cells in the presence of complement [64], a post mortem study of PEM/SSN and anti-Hu antibodies showed only weak complement deposition in the CNS of affected individuals [22]. Furthermore, two other studies failed to show any toxic effects of purified anti-Hu IgG on Hu expressing cell lines in the presence or absence of complement [72,73], and the study of Hormig and Lieberman [72] also failed to demonstrate antibody-dependent cell-mediated cytotoxicity.

In the absence of a definitive role for antibody-mediated neurological dysfunction, the finding of CD8 + T lymphocytic infiltration of neurological tissues at post mortem has prompted investigation of cytotoxic T cell mechanisms that could potentially lead to neurological dysfunction. An over representation of certain Vβ gene families in the predominantly CD8 + T lymphocytic brain infiltrates suggests that an antigen-driven oligoclonal cytotoxic T lymphocyte (CTL) response plays a role in the pathogenesis of an anti-Hu associated PEM/SSN [74]. However, as yet no data has been obtained regarding antigen-specificity of these CTLs. In contrast it has been possible to demonstrate expanded populations of memory and effector CTLs that recognize HLA-A2.1 restricted peptide epitopes derived from the Yo antigen in peripheral blood lymphocytes derived from five patients with anti-Yo antibodies and PCD [75,76]. However, whether CD8 + T lymphocytes specific for epitopes derived from the Yo antigen have a role in the pathogenesis of paraneoplastic cerebellar degeneration remains to be established. CD8 + T lymphocytes can be detected in the CSF of patients presenting with acute cerebellar dysfunction and anti-Yo antibodies [76] and future studies with peptide-MHC class I tetramers should enable the antigen-specificity of these T lymphocytes to be determined.

Do immune mechanisms lead to retardation of tumour growth in patients with PNS?

It has long been argued that an immune response against the cancer in patients with PNS leads to retardation of tumour growth. The epidemiological study of Maddison et al. [77] in which 15 SCLC patients with LEMS showed improved survival when compared with 15 SCLC patients without LEMS, with the two groups being matched for the extent of tumour spread, suggests anti-VGCC antibodies could inhibit tumour growth. In a smaller study of neuroblastoma patients, four patients with stage IV neuroblastoma and anti-Hu antibodies had a median survival of 86 months compared with 28.5 months in stage-matched controls [26]. However, much of the other evidence arguing for immune-mediated tumour surveillance is anecdotal. For example, the report of radiological regression of lung tumours in two patients with anti-neuronal antibodies and a PNS [78] does not represent definitive evidence of immune-mediated tumour destruction, since tumour necrosis due to the tumour outgrowing its vascular supply is just one of a number of non-immunity explanations for this phenomenon.

Although SCLC is usually confined to the mediastinum in patients with PEM/SSN and anti-Hu antibodies [8], and on occasions SCLC is only diagnosed with a careful post-mortem examination, it could be argued that this finding represents a lead-time effect arising due to the neurological syndrome resulting in earlier tumour diagnosis. However, it should be noted that 16% of SCLC patients develop an anti-Hu antibody response in the absence of neurological dysfunction and in one study positive anti-Hu antibody status, in the absence of neurological dysfunction, was a strong predictor of complete response to chemotherapy and was associated with longer survival [79]. Furthermore, HuD
DNA vaccination of mice leads to significant inhibition of the growth of an implanted neuroblastoma cell line that constitutively expresses HuD (even though the animals show no evidence of neurological dysfunction) [80]. Examination of the inflammatory cell infiltrates of the implanted tumours revealed increased numbers of CD8+ T lymphocytes [80]. Coupled to the observation that SCLCs associated with PEM/SSN continue to express MHC class I antigens [26], this raises the possibility that CTLs specific for HuD epitopes could result in SCLC destruction in this patient group.

Albert et al. [75] have observed that Yo-expressing HeLa cells induced to undergo apoptosis can be used to prime a cytotoxic T cell response against HLA-A2.1 restricted epitopes derived from the Yo antigen in vitro, but it remains to be established whether this response is important in leading to tumour immunity in vivo. Although Yo-expressing primary tumours associated with PCD and anti-Yo antibodies may contain a CD8+ inflammatory infiltrate [32], in the majority of reported cases the tumour lymphocytic infiltrate is predominantly composed of plasma cells [29]. In one recent study of 15/18 patients with PCD, anti-Yo antibodies and gynaecological disease had metastasic disease at presentation and progression of the cancer was the cause of death in just over half of the cases [9].

Therefore, it is important to recognize that there are many ways in which a tumour can evade immune surveillance by CTLs (For a review see [81]). Not only can tumours produce immunosuppressive products, but tumours can also down-regulate those processes that lead to peptide epitope presentation by MHC class I molecules. Furthermore, the study of Furneaux et al. [34], which examined aberrant Yo expression by tumours, revealed that Yo expression was not uniform throughout the tumour and that in some cases the Yo antigen was detectable in as few as 10% of tumour cells. Therefore, in such cases only 10% of cells are potential targets for CTLs specific for epitopes derived from the Yo antigen. This latter observation is of potential interest since it might be predicted that if the anti-Yo immune response does result in tumour destruction then metastatic lesions in patients with PCD and anti-Yo antibodies are likely to be derived from tumour cell populations that do not express the Yo antigen. This hypothesis is supported by the finding that Yo antigen expression could be detected in a primary oesophageal cancer but not in a cerebral metastasis derived from a patient with anti-Yo antibodies [32].

**What determines whether an individual will develop a PNS?**

If the immune system is to be manipulated in order to establish successful tumour immunotherapy it is critical to obtain an understanding of the natural tumour autoimmunity that appears evident in at least some individuals with PNS. It is proposed that aberrant neuronal antigen expression is responsible for the development of PNS. However, Yo antigen expression is frequently detected in ovarian, breast and gynaecological malignancies and is not just restricted to those tumours associated with PCD [33]. Similarly, only a small proportion of SCLC patients develop LEMS or anti-Hu antibody associated PEM/SSN, yet VGCCs and Hu antigens are expressed by virtually all SCLCs [19,82]. It has been suggested that development of the anti-Hu immune response occurs in association with SCLCs that express mutated Hu antigens, but gene analysis in SCLCs associated with anti-Hu antibodies and PEM/SSN has failed to detect mutations in the HuD gene [83] and it seems that the immune system is activated by tumours that express native antigen. Therefore, determining the role of antigen-presenting cells and T-helper lymphocyte responses should prove critical in our understanding of the interactions that occur between the immune system and the tumour in patients with PNS.

Currently we are still in the very early stages of investigating T-helper lymphocyte responses to paraneoplastic antigens. Benyahia et al. [84] demonstrated HuD-specific proliferation of peripheral blood lymphocytes in ten patients with anti-Hu antibodies and PEM/SSN that was associated with an increase in the interferon-γ/interleukin-4 ratio, and further studies need to be undertaken to determine the relevance of this finding to disease pathogenesis. In contrast, Yo antigen stimulation of peripheral blood lymphocytes from four patients with PCD and anti-Yo antibodies did not result in T cell proliferation or cytokine production [85]. Thus, it is apparent that further research is required if we are to advance our understanding of the processes involved in activating the anti-neuronal immune response.

Finally, the role that the blood–brain barrier may have in protecting individuals from developing PNS also requires further investigation. The peripheral nervous system, in particular the dorsal root ganglia and neuromuscular junction, are considered to be afforded less immunoprotection than the CNS and the commonest PNSs affect the neuromuscular junction (LEMS) and dorsal root ganglia (anti-Hu antibody associated SSN) [15]. Furthermore, 16% of patients with SCLC develop an anti-Hu antibody response without evidence of neurological dysfunction [79]. Those patients with PEM/SSN tend to have a higher titre of antibody, but similar titres of anti-Hu antibodies can occur in neurologically normal individuals [86]. Since there is no difference in antibody epitope reactivity between the anti-Hu+ with PEM/SSN and anti-Hu+ without PEM/SSN groups [86] other immune factors and/or factors that influence the ‘permeability’ of the blood–brain barrier may influence the development of neurological dysfunction.

Manipulation of the animal model of experimental allergic encephalomyelitis, has shown that CNS peri-
vascular accumulation of peripherally-administered activated T cells resulted in focal leakiness of the blood–brain barrier which was sufficient to allow entry of peripherally circulating antibodies capable of causing demyelination to enter the CNS [87]. Whilst the majority of paraneoplastic anti-neuronal antibodies appear to be devoid of pathogenic effects, the capability of T cell responses to compromise the blood–brain barrier integrity to peripherally active immune responses in patients with PNS needs to be explored further.

CONCLUSION

PNSs are a heterogeneous group of disorders that arise as non-metastatic complications of cancer and are believed to have an autoimmune pathogenesis. At least two paraneoplastic syndromes, LEMS and PCD associated with anti-GluR antibodies, are mediated by anti-neuronal antibodies with pathogenic actions. However, a pathogenic role for other paraneoplastic antibodies that react with intracellular neuronal antigens has yet to be demonstrated, even though intrathecal synthesis of these antibodies can be demonstrated. Therefore, much of the current research into the effector mechanisms that result in neurological dysfunction is aimed at investigating cytotoxic T cell responses to intracellular neuronal antigens. Nevertheless, the serum and CSF anti-neuronal antibody responses detected in patients with PNSs are often oligoclonal [47] and as yet undefined antigens may prove to be the target of pathogenic immune responses. Since in the majority of cases PNSs are poorly responsive to treatment with existing immunotherapies [9,88], gaining a better understanding of the immunopathogenesis of these severely debilitating disorders is imperative in order to allow development of better immunotherapeutic management strategies. Furthermore, an improved understanding of the interaction between the immune system and tumour in individuals with paraneoplastic neurological disorders may also help advance immunotherapeutic approaches to cancer treatment in general.

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