Letters

Anti-Purkinje cell cytoplasmic and neuronal nuclear antibodies aid diagnosis of paraneoplastic autoimmune neurological disorders

Sir: Confusion has arisen in the literature concerning the association of serum anti-neuronal antibodies and paraneoplastic neurological disorders,[1] due partly to lack of standardisation of serological nomenclature and methodology, and partly to inadequate definition of antibody specificities. This is unfortunate, because clinical-serological correlations obtained in our recent screening for two distinct antibody specificities in a clinical laboratory indicate that simple indirect immunofluorescence testing[2] is a valuable aid in establishing the paraneoplastic nature of certain neurological disorders. When positive, the procedure described below has the potential for enabling 1) inapparent cancer to be detected early enough to possibly effect a cure, and 2) anti-tumour treatment to be initiated before neurological impairment is severely disabling.

For clinical purposes, it is our practice to screen sera for anti-neuronal antibodies at 1:60 dilution, using unfixed frozen sections (6 μ) of cerebellum (human, and mouse or guinea pig) and fluoresceinated polyvalent anti-human IgG[2]. Two distinctive patterns of immunostaining are sought. Positive sera are titrated, and those with nuclear staining are tested additionally with fluoresceinated anti-human IgG on both neuronal and non-neuronal substrates. The two distinctive patterns are as follows:

1 *Anti-Purkinje cell cytoplasmic antibodies (PCAb).* These antibodies react in a striking and characteristic pattern (fig 1) with the cytoplasm of cerebellar Purkinje cells, and faintly with molecular neurons.[2,3] We have identified a total of 21 seropositive patients so far, 13 prospectively (that is, with neurological signs but before cancer was detected or found to be recurrent). The patients were all female, postmenopausal, and had signs of subacute cerebellar degeneration. All were found at follow up to have active cancer (15 ovarian, 2 Fallopian tube, 1 endometrial, 2 breast and 1 lymphoma). Of six seropositive patients with no signs of cancer at the initial examination and negative CT scanning of the abdomen and pelvis, one was found seven months later to have metastatic adenocarcinoma in a biopsied inguinal lymph node,[2] and all five subsequent patients were found, at exploratory laparotomy, to have foci of metastatic ovarian adenocarcinoma in the pelvis. In 12 of the 21 seropositive cases, PCAb of IgM or IgA class were identified in addition to IgG, but at a lower titre. All cases had IgG PCAb of high titre (endpoint dilutions 1/1000–1/128,000). We have not found PCAb in sera of patients with ovarian cancer without PCD (n = 39), with non-neoplastic cerebellar degenerative disorders (n = 20), nor with cerebellar degeneration associated with small cell lung carcinoma (SCC) (n = 11).

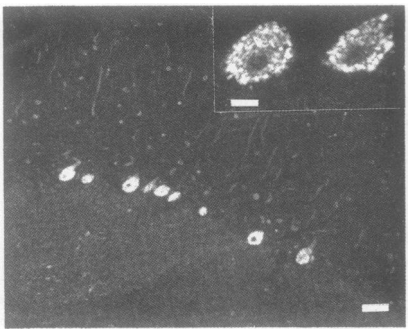

Fig 1 *Anti-Purkinje cell cytoplasmic antibodies (PCAb) stain Purkinje cell bodies and dendrites, and molecular layer neurons in a section of normal human cerebellum. Metastatic ovarian adenocarcinoma was found at exploratory laparotomy in this 60 year old woman with a six week history of progressive cerebellar dysfunction who had no clinical or abdominal CT evidence of cancer. (Serum diluted 1:480; bar indicates 50 μm). Inset: staining of Purkinje cell cytoplasm by PCAb is characteristically coarsely granular. (Same serum diluted 1:960; bar indicates 15 μm).*

Other North American researchers,[4,5] agree that PCAb are highly correlated with paraneoplastic cerebellar degeneration (PCD) occurring in association with carcinoma of the ovary. Although PCAb bind to cytoplasmic components of many neuronal types,[2,4] (VA Lennon unpublished observations), we consider the terminology "PCAb" appropriate for the clinical definition of this serological specificity, because symptoms and signs of cerebellar dysfunction dominate the neurological presentation in most seropositive patients, and because immunostaining in the cerebellum is most striking in Purkinje cells.

2 *Anti-neuronal nuclear antibodies (ANNA)* The ANNA specificity is distinguished from non-organ-specific anti-nuclear antibodies (ANA) by selective binding to neurons.

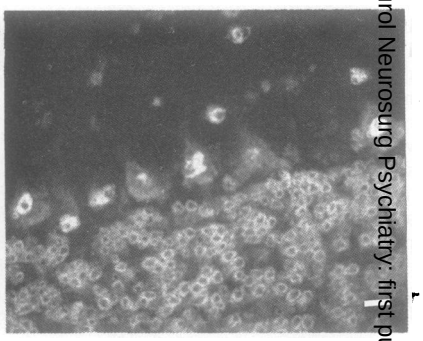

Fig 2 *Anti-neuronal nuclear IgG antibodies (ANNA) stain discrete nuclear elements in Purkinje cells of a guinea pig cerebellum. Heterochrometin is unstained. Characteristically, the cytoplasm is faintly stained. Peripheral nuclear elements of granular and molecular layer neurons are also stained. Non-neural cells and tissues were not stained. Small cell lung carcinoma was found in this 61 year old man who presented with a severe neuropathy (predominantly autonomic). (Serum diluted 1:200; bar indicates 15 μm).*

ANNA (fig 2) react with both nuclear and cytoplasmic elements of cells in the central and peripheral nervous system. In large neurons, like Purkinje cells, nuclear staining is striking. Valid identification of a positive result requires that testing for ANA also be performed (for example, using a substrate of mouse stomach or kidney), and be negative or of significantly lower titre. So far we have identified IgG ANNA in fifteen patients, three with subacute sensory neuronopathy with SCC, four with predominantly autonomic neuropathy with SCC, six with mixed sensory-autonomic or sensorimoter neuropathy with SCC, one opsoclonus-myoclonus and sensorimotor neuropathy with SCC, and one a mixed sensorimotor neuropathy with a history of tobacco abuse and bilateral mastectomies for breast carcinomas.

ANNA were initially described in patients with the syndrome of sensory neuronopathy with SSC,[6-8] and have subsequently been reported in numerous other SCC-associated paraneoplastic syndromes involving the central and peripheral nervous system.[9] However, the spectrum of clinical disorders associated with the ANNA marker is not yet known. We have not found IgG ANNA in normal subjects, in patients with peripheral neuropathies unassociated with cancer (n = 10), with Parkinson's disease (n = 25), with SCC without evidence of neurological disease (n = 20), nor in patients with the

Lambert-Eaton myasthenic syndrome (n = 14, three had coexisting cerebellar degeneration and six had SCC). It is noteworthy that non-organ-specific ANA were found in 33% of patients with Lambert-Eaton syndrome who had cancer (n = 50). Patients with this syndrome have a variety of autoantibodies.[10] This emphasises the necessity of retesting and titrating any serum that is positive for neuronal nuclear reactivity on substrates of neural and non-neural tissues side-by-side to confirm neuronal specificity.

Immunoblot criteria have been used by some researchers to classify different types of anti-neuronal antibodies.[9 11] However, our experience with the immunofluorescence screening procedure described above validates the clinical usefulness of a positive result obtained with this simple test. Seronegativity does not exclude the presence of a malignant tumour. Occasionally atypical patterns of cerebellar staining are encountered, that is, not strictly fitting either of the two patterns described. At present no attempt is made to assign them clinical significance.

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Crying and laughing after brain damage: a confused nomenclature

Sir: Brain damage is commonly associated with abnormalities of emotional expression. This has long been recognised[1] and such changes have excited the interest of both neurologists[2] and psychiatrists.[2] Episodes of crying and unconstrained outbursts of laughter are most often described in connection with stroke,[3] multiple sclerosis,[4] cerebral tumour,[2] and motor neuron disease[5] but they are not specific to any particular neurological disorder and can follow most causes of brain damage.[6] Possible pathophysiological mechanisms have been discussed,[6] but their exact nature remains obscure. Lesions are usually multiple and bilateral and involve the corticobulbar tracts.[2] These changes are often distressing to patients and their carers and they can contribute to difficulties in management and diagnosis. There has been no systematic study of the phenomenology of these expressions and we know little about their physical or psychological correlates. What is striking about the existing literature is the multiplicity and confusion of the terminology. Communication would be facilitated by a consensus as to the most appropriate nomenclature. In this letter I will discuss the relative merits of existing terms and propose one of them as a candidate for future use.

Authors have often used different terms interchangeably, for example, "involuntary crying", "pathological emotionality", and "forced crying" may be construed as synonymous in describing the same phenomenon. In contrast, some authors use separate terms to refer to what they consider to be distinct phenomena, for example Poeck[6] makes a distinction between "pathological crying and laughing" on the one hand and "emotional lability" on the other. Some terms include reference to aetiology, as in "pseudobulbar affect" and "organic emotionalism", or to severity, as in "emotional incontinence." Others are more descriptive or judgmental as in "spasmodic crying," "inappropriate crying" and "pathological affect".

Some of these terms are clearly unsuitable or inadequate and should be abandoned. "Pseudobulbar affect", for example, can have only a limited application since the majority of patients with brain damage and episodes of crying or laughing will never have so called pseudobulbar signs. "Involuntary crying" alludes to absent or diminished control over the expressions but crying in non-brain damaged people is in a sense "involuntary" so the term is redundant. "Inappropriate crying or laughing" implies (wrongly) that it is possible to judge the appropriateness of an emotional expression under given circumstances. Also, this term detracts from making an effort to understand why the expression might be appropriate in any particular situation. "Emotional incontinence," although it invokes a vivid image of patients with extreme and uncontrollable episodes of crying or laughter (or both), has too many excretory connotations for regular use!

We are left with the terms: "pathological crying or laughing", "emotional lability" and "emotionalism". The first is the least satisfactory because of the problem of deciding what is "pathological"—is the emotional expression itself "pathological" because it (sometimes) appears different in some way to "normal" or does "pathological" refer to the underlying brain damage? "Emotional lability" is attractive in that it conveys meaning without being judgmental or confusing. The shortened form "lability" is also convenient for day to day use. However, the term is used widely in psychiatry to describe emotional states that are quite distinct from those seen in brain damage. The term "emotionalism" has been less widely used but unlike "emotional lability" it is not also employed to describe unrelated phenomena. It has the added advantages of being a single word, free of inaccurate aetiological inference and also non-judgmental.

In conclusion, the existing nomenclature for the abnormalities of emotional expression following brain damage is confused and inadequate. Consistency is required in order