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Review

Onconeural antigens and the paraneoplastic neurologic disorders: At the intersection of cancer, immunity, and the brain

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ABSTRACT **Paraneoplastic neurologic disorders (PNDs) are believed to be autoimmune neuronal degenerations that develop in some patients with systemic cancer. A series of genes encoding previously undiscovered neuronal proteins have been cloned using antiserum from PND patients. Identification of these onconeural antigens suggests a reclassification of the disorders into four groups: those in which neuromuscular junction proteins, nerve terminal/vesicle-associated proteins, neuronal RNA binding proteins, or neuronal signal-transduction proteins serve as target antigens. This review considers insights into basic neurobiology, tumor immunology, and autoimmune neuronal degeneration offered by the characterization of the onconeural antigens.**

The unusual nature of a rare group of diseases, the paraneoplastic neurologic disorders (PNDs), offers the opportunity of developing insights into tumor immunology, autoimmune neurologic disease, and basic neurobiology. PNDs are believed to be autoimmune disorders that arise when systemic malignancies express proteins that are normally made only in neurons (onconeural antigens). A critical insight into the pathogenesis of the disorders was the recognition by Jerome Posner and coworkers that PND patients harbor high-titer antibodies in both their serum and spinal fluid that recognize apparently identical antigens in Western blots of normal brain and PND tumor tissues (refs. 1–5; for review see ref. 6). The presence of PND antibodies correlates with effective antitumor immunity (7–11), and their detection by Western blot both predicts the presence of specific underlying malignancies and definitively establishes the PND diagnosis (6, 12). Over the past several years PND antibodies have been used as reagents to clone and characterize a number of target PND antigens, allowing studies of a series of previously undiscovered neuronal proteins.

Antitumor immunity in PND patients became evident only by its association with severe neuronal degeneration. Most PND patients who present with neuronal degenerations are unaware that they harbor an occult malignancy (most commonly breast, ovarian, or small cell lung tumors); in rare instances, malignant neoplasms have been documented to vanish without treatment after the onset of neurologic disease (10, 13). Within the nervous system, nearly any group of neurons can be targeted in PND, including those of the limbic system, retina, cerebellum, brainstem, spinal cord, and dorsal root ganglia (Table 1). The immune system typically targets a single onconeural antigen in PND tumors, giving rise to a discrete set of neurologic symptoms [only very rarely have two distinct antigens been found to be targeted, leading to two superimposed neurologic syndromes (14–16)]. These observations suggest a model (considered below) in which the expression of neuronal antigens in tumor cells leads to an immune response which suppresses tumor growth but leads to the destruction of neurons (Fig. 1). It should be noted, however, that this model rests solely on clinical data; to date, with the exception of the PNDs of the neuromuscular junction (NMJ), no animal model for these disorders has been established.

In addition to the significance that identification of the onconeural genes has for tumor immunology and autoimmune neurologic disease, these genes also encode proteins that are likely to be of unique importance to neurons. The model for the pathogenesis of the PNDs (Fig. 1) suggests that the normal expression of onconeural antigens is exquisitely restricted to immunologically privileged cells, allowing their recognition as foreign antigens when ectopically expressed in tumors. A corollary to this hypothesis is that as target antigens in neuronal degeneration, onconeural antigens are expressed in neurons; the data reviewed below suggest that in most cases onconeural genes are expressed exclusively in neurons. Thus the cloning of these genes using PND antisera provides an exceptional opportunity to study neuron-specific function. Finally, whereas the function of onconeural antigens is just beginning to be explored, the observation that specific tumor types express specific onconeural antigens (Table 1) suggests that the regulation of expression, and perhaps more importantly, the function of these proteins may have biologically important roles in tumor cells and neurons.

Classification of the PNDs

The PNDs have traditionally been classified according to the clinical neurologic symptoms by which they were identified. Although recent work suggests that additional PNDs exist, four well-defined clinical syndromes initially led to the identification of four sets of antibodies and to the cloning of genes encoding target antigens (Table 1). These are (*i*) paraneoplastic cerebellar degeneration, in which women with breast or ovarian tumors harbor an antibody termed Yo that recognizes a 52-kDa antigen present in the tumors obtained from these patients as well as in cerebellar Purkinje neurons (6, 9, 17); (*ii*) paraneoplastic blindness (cancer-associated retinopathy), in which patients with small cell lung cancer harbor antibodies against a 23- kDa antigen present in the tumor cells and photoreceptors (18); (*iii*) paraneoplastic opsoclonus-myoclonus-ataxia (POMA), a motor disorder manifested by dysfunction of a subset of brainstem, spinal cord, and cerebellar neurons, in which patients with breast, fallopian, or lung tumors harbor an antibody that recognizes a 55-kDa antigen present in their tumor specimens and neuronal nuclei (19, 20); and (*iv*) paraneoplastic encephalomyelitis/sensory neuronopathy (PEM/SN), a diffuse group of neurologic disorders manifested by symptoms of sensory loss, memory loss, cerebellar, brainstem, motor, or autonomic dysfunction that typi-

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Abbreviations: PND, paraneoplastic neurologic disorder; NMJ, neuromuscular junction; POMA, paraneoplastic opsoclonus-myoclonus-ataxia; MG, myasthenia gravis; LEMS, Lambert–Eaton myasthenia syndrome; AChR, acetylcholine receptor; SMS, stiff-man syndrome; GAD, glutamic acid decarboxylase; n-RBP, neuron-specific RNA-binding protein; CNS, central nervous system; MHC, major histocompatibility complex.

Table 1. PNDs defined by autoimmune antibodies

Neurologic syndrome			
Paraneoplastic name	Phenotype	Tumor	Antibody
Cerebellar degeneration	Pan-cerebellar dysfunction (Purkinje cell)		
Typical		Breast, ovarian	Yo
Atypical		None identified*	β-NAP
Paraneoplastic opsoclonus myoclonus ataxia (POMA)	Motor (brainstem, spinal, cerebellar)	Breast, fallopian	Ri
Encephalomyelopathy/ sensory neuropathy (PEM/SN)	Multifocal Sensory Limbic Motor Autonomic Cerebellar	Small cell lung	Hu
Cancer-associated retinopathy	Blindness (photoreceptor)	Small cell lung	CAR
Stiff-man syndrome (SMS)	Motor (spinal interneuron?)	Breast	Amphiphysin
Paraneoplastic NMJ	Motor		
LEMS		Small cell lung	Various†
MG		thymoma	α-AChR

*Expressed in neuroectodermal tumor lines.
†See text.

cally progress into a multisystem neuronal degeneration (14), in which patients with small cell lung cancer harbor an antibody termed Hu that recognizes 35- to 40-kDa antigens present in neuronal nuclei and small cell lung tumors (21). In addition to these paraneoplastic syndromes, two disorders involving the NMJ, myasthenia gravis (MG), and the Lambert–Eaton myasthenic syndrome (LEMS), are frequently associated with underlying malignancy, and are associated with antibodies to the acetylcholine receptor (AChR) and the presynaptic calcium channel, respectively.

In this article I will review the current understanding of the nature of the onconeural antigens, reclassifying the PNDs into four discrete categories according to the nature of the target antigens (Table 2). A question that arises from this classification is whether proteins within a group share common features that render them susceptible to antineuronal immunity or that

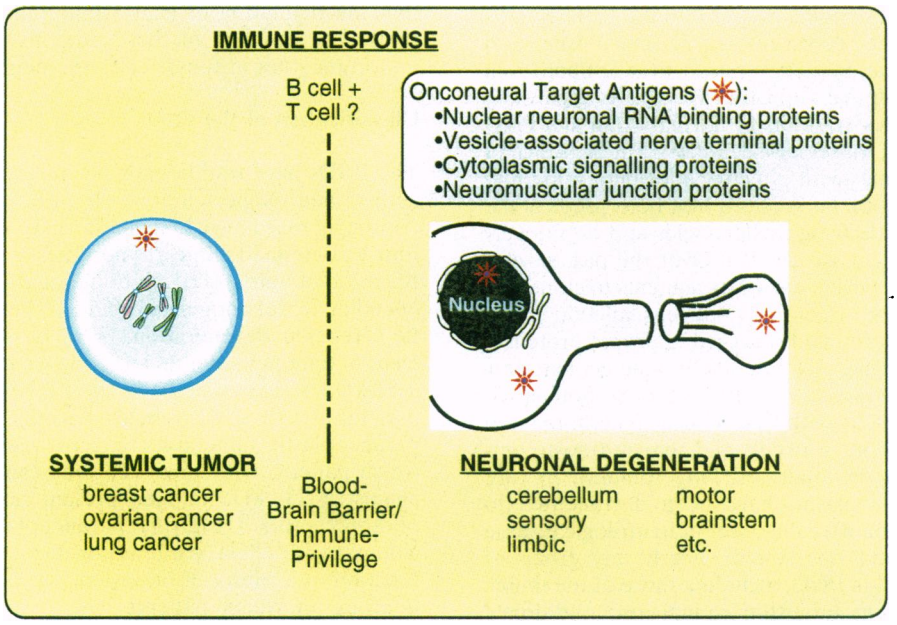

FIG. 1. Model for the pathogenesis of the PNDs. The paraneoplastic disorders are believed to initiate when solid tumors present outside of the nervous system express proteins that are normally made only in neurons. Perhaps in part because of the blood-brain barrier and/or the immunologically privileged state of neurons, the immune system recognizes the neuronal protein as foreign when ectopically expressed in tumor cells. A low level immune response is generated, which is associated with effective antitumor immunity. A second event is then postulated in which the ongoing antitumor immune response becomes competent to recognize neurons normally expressing the paraneoplastic antigen; such an event could either be disruption of the blood-brain barrier (e.g., cytokine mediated) or a change in the nature of the immune cells themselves. This establishes an autoimmune neurologic degeneration, which brings patients to clinical attention. There are two features of this model supported by data discussed in this review that are particularly relevant to neurobiology. (*i*) The model postulates that the effective immune recognition of neuronal antigens in tumor cells arises because the target antigens are normally exclusively expressed in neurons, both during development and during adulthood. If they were expressed outside of the immunologically privileged nervous system, they would be recognized as self-antigens and would not produce immunity. Thus, PNDs provide a means to study neuron-specific function through the identification of neuron-specific genes. (*ii*) The expression and function of the onconeural antigens, while not known, is of potential interest because specific tumor types selectively express specific neuronal proteins. This suggests that the regulation of expression and function of the onconeural genes may be particularly revealing to examine in tumor cells and neurons.

Table 2. Four categories of onconueral antigens

Antigen	Syndrome	Function
Nerve-terminal and vesicle-associated proteins		
Amphiphysin	SMS	Vesicle-associated; dynamin and AP2 interaction
β-NAP	Cerebellar degeneration*	Vesicle coat protein (related to AP2)
Glutamic acid decarboxylase	SMS*	Synthesizes γ-aminobutyric acid; vesicle associated
Synaptotagmin	LEMS	Vesicle-associated protein; AP2 interaction; Ca^{2+} sensor
Neuron-specific RNA-binding proteins		
Nova family (≥2 members)	POMA	KH motif RBPs; homologies to hnRNP-K, FMR-1, MER-1, PSI
Hu family (≥ 4 members)	PEM/SN (multifocal)	RRM motif RBPs; mammalian homologues of elav
Putative neuronal signalling proteins		
cdr2 (Yo)	Cerebellar degeneration	Cytoplasmic protein; N-terminal amphipathic helix-leucine zipper
Recoverin	Blindness	cGMP-gated signal transduction
Neuromuscular junction proteins		
Presynaptic Ca^{2+} channel (β-subunit)	LEMS	Signal transduction
AChR α-subunit	MG	Acetylcholine binding subunit of receptor

*No tumor association.

correlate with their immunity in tumor cells. The cloning and characterization of onconeural antigens will allow for an examination of whether similarities in protein function, cellular location, antigenicity, susceptibility to functional disruption by antibody, or other features determine similar mechanisms of paraneoplastic neurologic disease. These questions are illustrated by considering each category of PND antigens individually.

Neuromuscular Junction Antigens

Historically, the first paraneoplastic antigen identified was the α-subunit of the nicotinic AChR, which is expressed at the postsynaptic motor endplate of the NMJ and is the target antigen in MG. Although MG is frequently thought of as an autoimmune neurologic disease, the AChR is expressed in thymomas associated with MG (22, 23), suggesting that in at least some cases MG is a paraneoplastic syndrome. In LEMS, a second PND involving the NMJ, patients harbor antibodies against components of the presynaptic motor endplate, and 60% of LEMS patients are found to have small cell lung cancer. *In vitro*, LEMS antibodies lead to impaired calcium flux (24, 25) and impaired acetylcholine release (26) from the presynaptic motor neuron. Unlike MG, a single autoantigen has not been identified in LEMS. Despite reports that many (27) or all (25) LEMS antisera bind to P/Q type voltage-gated presynaptic calcium channels, the specificity of such antibodies is uncertain, given that they can also be found in normal individuals or patients with unrelated neurologic disorders (25). A subset of LEMS patients harbor antibodies against other presynaptic nerve terminal components (see below), but the role of such antibodies in LEMS is uncertain.

MG and LEMS have several features that distinguish them from the other paraneoplastic disorders discussed here. First, the localization of the neurologic dysfunction at the NMJ places the site of immune attack at the border between neurons and the rest of the body, and the antigens under attack appear in extracellular localizations accessible to circulating antibodies. Second, autoantibodies play a demonstrated role in the pathogenesis of autoimmunity at the NMJ. For example, the major immunogenic epitope of the AChR is a region of the extracellular domain of the receptor susceptible to antibody attack (28, 29), and anti-AChR antibodies passively transfer the myasthenic syndrome to animals (28, 30). Although a single dominant epitope has not been defined, mice treated with LEMS IgG have distorted active zones at the presynaptic junction (31) and LEMS antisera passively transfers the disease to animals (26, 32). Finally, both MG and LEMS patients, unlike other PND patients, benefit from plasmapheresis or immunosuppressive treatments that suppress B-cell function (33, 34).

In contrast to the NMJ target antigens, the PND antigens discussed below appear to be neuron-specific, intracellular proteins, and, where tested, the generation of PND antibodies in animals has failed to produce disease (35, 36). These observations suggest that paraneoplastic neurologic disorders involving the NMJ may have a fundamentally different pathophysiology from other PNDs. Whereas a mechanism of disease involving neuronal antibody uptake could be relevant for the remaining PNDs (see below), a role for other immune mechanisms (e.g., cytotoxic T lymphocytes or other cytolytic killer cell activity) seems particularly important to consider in these disorders, given their association with effective antitumor immunity and the intracellular localization of the target antigens.

Nerve Terminal/Vesicle-Associated Antigens

Recent studies suggest that intracellular vesicle-associated proteins in the presynaptic nerve terminal form a distinct group of target antigens in both autoimmune and paraneoplastic neurologic disease. The first protein identified in this context is a target antigen in stiff-man syndrome (SMS; ref. 37), the enzyme glutamic acid decarboxylase (GAD), which converts glutamate to GABA. SMS is an autoimmune neurologic disorder (not generally associated with cancer) characterized clinically by motor symptoms consistent with GABAergic blockade, and the clinical symptoms are specifically ameliorated by GABA agonists. One mechanism for the pathogenesis of SMS consistent with these observations is that the autoantibody specific for GAD blocks the activity of this enzyme. GAD exists in the nerve terminal as a vesicle-associated soluble protein (38), suggesting that if antibody were taken up in the nerve terminal and able to gain access to the soluble proteins there, it might interfere with GAD protein function, much as antibodies microinjected into neurons *in vitro* are able to disrupt the function of vesicle membrane proteins in the nerve terminus (39).

Following the discovery of anti-GAD antibodies in SMS, a series of antibodies against intracellular presynaptic nerve

terminal proteins have been identified as autoantigens in neurologic disorders. A variant of SMS that develops in patients with breast cancer is associated with a paraneoplastic antibody against the nerve terminal/vesicle-associated protein amphiphysin (40). Amphiphysin appears to play a role in synaptic vesicle endocytosis in the nerve terminal by binding to the vesicle coat protein adaptor AP2 and dynamin (41, 42). Similarly, antiserum from a patient with an autoimmune cerebellar degeneration (in whom no tumor was identified) was used to characterize and identify a novel vesicle coat protein termed β-NAP (43, 44). β-NAP protein and mRNA are present exclusively in neurons where the protein exists as a cytoplasmic pool that appears to be recruited onto a subset of vesicles in both the cell soma and the nerve terminal (43, 107). Finally, in LEMS, a number of presynaptic nerve terminal proteins have been identified as antibody targets, including synaptotagmin (45, 46), a vesicle-associated calcium channel sensor, and an intracellular (β) subunit of the presynaptic calcium channel (47).

Taken together, at least four vesicle-associated antigens present in the presynaptic nerve terminal have been clearly identified as autoimmune or paraneoplastic target antigens (Table 2). The common cellular localization of this set of antigens suggests that they may share a particular vulnerability to immunologic attack and/or disruption of function. It will be of interest to determine whether such selective vulnerability relates to a special susceptibility of nerve terminal proteins to inhibition of protein function following antibody exposure, or to other mechanisms such as selective antigen presentation and/or protein immunogenicity.

Neuron-Specific RNA-Binding Proteins

A second discrete class of neuronal proteins to be identified as target antigens in PNDs are neuron-specific RNA-binding proteins (n-RBPs). Two distinct families of proteins fall into this category. The Nova antigens are homologous to a newly described class of RNA- binding proteins characterized by the presence of KH RNA-binding motifs (48). The Hu antigens comprise a second set of RNA-binding proteins characterized by the presence of three canonical ~80 amino acid RNA recognition motifs (48). The Nova and Hu proteins share several common features: (*i*) they appear to be expressed exclusively in neurons from early in development through adulthood, (*ii*) they are predominantly expressed as nuclear proteins, and (*iii*) they harbor suggestive sequence homologies with RNA-binding proteins involved in the regulation of alternative splicing.

The Nova-1 gene was identified using high-titer antisera from a patient with POMA and breast cancer; serum reactivity with Nova-1 fusion protein is diagnostic for the disorder in adults, and serves to prompt physicians to search for the presence of occult breast, gynecologic, or lung tumors. Nova-1 expression is tightly restricted; the mRNA is only detectable in brain on Northern blots (19), and protein expression is restricted to the nucleus and, to a lesser degree, cytoplasm of neurons (49). Moreover, immunohistochemical and *in situ* hybridization studies reveal that Nova-1 expression is restricted to neurons of the central nervous system (CNS) throughout mouse development, where its expression is tightly restricted to the diencephalon, brainstem, and spinal cord (19, 49).

These observations are consistent with the paradigm of PND pathogenesis presented in this review; the restricted expression of the Nova antigen and mRNA to the CNS during development and into adulthood suggest that the protein is normally sequestered from the immune system and thereby potentially immunogenic in tumor cells. The distribution of Nova-1 mRNA within the brain correlates in an approximate way with the motor syndrome (involving an undefined mix of brainstem, cerebellar, and/or spinal motor neurons) present in POMA patients (although these patients rarely develop a more diffuse encephalopathy; see ref. 50). This correlation has been complicated by the observation that POMA antisera used in immunohistochemical stains under nonstringent fixation conditions recognizes all CNS neurons (19, 20, 51); this discrepancy may partly be explained by the identification of at least one and perhaps as many as three additional Nova genes (ref. 19 and Y. Y. Yang, G. L. Yin, and R.B.D., unpublished observations) whose products are reactive with native POMA antisera. In addition, POMA antisera identifies a minor band of 70–80 kDa which has not been characterized. The specificity of neuronal dysfunction seen in POMA might result from differential susceptibility of either subsets of Nova antigens or subsets of Nova-expressing neurons to autoimmune attack.

Sequencing of the Nova-1 gene revealed the presence of three repeated motifs homologous with the repeated KH domains present in the hnRNP K protein (19). KH motifs are also found in FMR-1, the product of the fragile-X gene (52), and in two RNA-binding proteins implicated in the regulation of alternative splicing in *Drosophila* and yeast, termed PSI (53) and MER-1 (54), respectively. The Nova-1 protein has the characteristics of an RNA-binding protein *in vitro* where it binds to RNA with the same sequence preference (to ribohomoguanosine) as FMR-1. One hallmark of POMA disease antisera (found in six of six samples) is that they specifically recognize an epitope that lies within the third Nova-1 KH motif. Interestingly, affinity-purified POMA antibodies completely abrogate the RNA-binding activity of the intact Nova-1 protein (49). This *in vitro* observation is reminiscent of the suggestion made for SMS; that autoimmune antibodies may not only bind to but disrupt the function of their target antigens, and suggests that POMA antibody might act to disrupt the activity of the Nova-1 RNA-binding protein in neurons.

Antibodies to the Hu antigens are associated with a diverse set of neurologic degenerative disorders. Neuronal dysfunction localizes most commonly to the dorsal root ganglia (in ~60% of Hu patients), but may relate to the cerebellum, brainstem, limbic system, motor neurons, or the autonomic nervous system, solely or as part of a multifocal disorder (14). Cloning of the Hu antigens using the patient's antisera originally yielded a single gene termed HuD that was found to encode a human homologue of the *Drosophila* elav protein (55). This connection was of importance because elav is a neuron-specific protein whose function is known to be essential for neurogenesis in *Drosophila* (56, 57), suggesting a potentially important role for HuD in mammalian neurobiology. Although no function has been determined for the Hu antigens, a target epitope in HuD, mapped using Hu disease antisera, localizes to the first two HuD RNA-binding domains (58). This suggests a possibility raised with the Nova antigens that antibody mediated disruption of the Hu RNA-binding activity might lead to neuronal death in patients with the neurologic syndrome.

Database alignments reveal that HuD is also highly homologous to the *Drosophila* RBP *sex-lethal*, primarily within the conserved RNA recognition motifs, but also to a significant degree in the sequence between them (55). This suggested a possibility that still has not been tested—that the Hu proteins may be involved in regulating alternative splicing within neurons—and spurred the cloning of additional family members by degenerate PCR and cDNA cloning. A total of four independent but highly homologous Hu genes have been identified that encode epitopes reactive with Hu antisera. Each of the Hu genes encode highly related n-RBPs, and each are alternatively spliced within their coding region.

Whether regional differences in the expression pattern of individual Hu genes or their spliced products correlate with the diverse neurologic symptomatology found in the Hu syndrome

is unknown. Immunohistochemical analysis (59) and *in situ* hybridization (60) performed with a probe from a conserved region of the Hu coding sequence demonstrate that Hu expression is restricted to neurons. *In situ* hybridization using gene-specific probes suggests marked variability in the developmental and tissue distribution of all four genes, in addition to confirming neuron-specific expression (H. J. Okano and R.B.D., unpublished data). Most patients with neurologic disease affecting predominantly one region of the nervous system ultimately develop a multifocal neurologic illness, dying from their neurologic disease an average of 7 months after the onset of symptoms. Thus, if the Hu autoimmune attack is initially directed to a single Hu gene product, it is possible that it ultimately becomes competent to recognize additional Hu family members.

In addition to the original HuD clone, the Hel-N1 (61), HuC (55, 62), and HuE (H. J. Okano and R.B.D., unpublished data) genes all encode members of the Hu family. Mapping of the mouse homologues of these four Hu genes reveals that they are clustered in pairs on two chromosomes, suggesting that they arose by gene duplication from a single common precursor (C. F. Fletcher, Copeland, N. G., Jenkins, N. A. and R.B.D., unpublished data). Biochemically, Hel-N1 has been shown to be an RBP in *in vitro* assays where it is able to bind to short stretches of uridylates and to AU-rich elements found in the 3′ untranslated regions of some mRNAs (60, 61). However, the *in vivo* significance of these observations is unclear, in part because the biologic significance of the AU-rich sequences found to bind Hel–N1 is uncertain (63, 64), and in part because AU-rich elements are involved in many aspects of RNA metabolism, including the regulation of splicing and mRNA stability (65).

Given the extensive homologies between RNA-binding proteins that regulate alternative splicing and the Nova and Hu n-RBPs, it is tempting to speculate that these n-RBPs regulate alternative splicing in neurons, although a role for the proteins in regulating neuronal mRNA stability, translation, or subcellular localization remains both possible and of great potential interest. In considering the possibility that n-RBPs regulate neuronal splicing, it is worthwhile recalling the role of sxl in development (for reviews, see ref. 66 and 67). During the sexual development of flies, the sxl protein acts as a binary switch; in the presence of sxl females develop, in the absence of sxl males develop. The mechanism of sxl action is through its role as a sequence-specific RNA-binding protein; by binding to a polypyrimidine tract upstream of exon 3 of the *tra* primary transcript, sxl displaces the constitutive splicing machinery (specifically, the RNA-binding protein U2AF; ref. 68), forcing U2AF to bind to a secondary polypyrimidine tract, leading to usage of an alternative splice acceptor and a *tra* transcript that encodes a functional protein. Ultimately, a series of alternative splice choices becomes established, resulting in the development of female flies. Since the discovery of neuron-specific and nonneuronal spliced forms of the calcitonin-CGRP primary transcript (69, 70), there has been speculation that neuron-specific RBPs mediate fundamentally different exon usage in neurons.

The identification of the onconeural n-RBPs is consistent with such speculation, and their complexity extends it in several ways. The onconeural n-RBPs include at least six and possibly more members, present in two different gene families; moreover, there is extensive alternative splicing within the coding regions of the n-RBP genes themselves (19, 55, 71), with the potential to generate ≥70 n-RBP protein variants. The expression patterns of individual genes suggests specificity of expression within individual sets of neurons (R. Buckanovich, Y. Y. Yang, H. J. Okano, and R.B.D., unpublished data). The clearest example to date is Nova-1, whose mRNA is expression is tightly restricted to subsets of neurons; it is absent from the neocortex and thalamus, and abundant in regions of the diencephalon, midbrain, and hindbrain (49). These observations suggest a role for n-RBPs that goes beyond the neuron-versus nonneuron binary switch suggested from the alternative splicing of calcitonin–CGRP, to a role in establishing unique characteristics of specific subsets of neurons. Since n-RBPs are targeted in adult neurologic syndromes, region-specific n-RBPs are also likely to be critical for the maintenance or function of sets of adult neurons. An attractive feature of the hypothesis that n-RBPs regulate splicing in neurons is that it suggests a means for the generation of diversity of neuronal function, using sets of n-RBPs to generate complexity from a limited size genome.

Neuronal Signal Transduction Proteins

A final set of onconeural antigens includes a group of two proteins with potential roles in signal transduction pathways. The first such protein identified was the paraneoplastic retinal degeneration antigen recoverin. Antisera from patients who became blind in the setting of small cell lung cancer were used to characterize and ultimately clone the gene encoding a 23-kDa antigen expressed in photoreceptors. This clone turned out to encode recoverin (72, 73), although it should be noted that other uncharacterized paraneoplastic retinal antigens may also exist (18). The identification of recoverin as an onconeural antigen is of interest given the possible role of this protein in cGMP signal transduction cascade in photoreceptors. Disruption of this phototransduction signaling pathway leads to photoreceptor degeneration (74, 75), suggesting that targeted disruption of recoverin by the PND immune response may be involved in the photoreceptor degeneration seen in these patients.

The most common paraneoplastic cerebellar degeneration syndrome, described in 55 patients in ref. 9, occurs in patients with breast or ovarian cancer who harbor an autoantibody termed Yo, which has been used by two groups to clone a target antigen termed cdr2. Reverse-transcription (RT)–PCR analysis of a single tumor specimen from a Yo positive patient revealed expression of the cdr2 gene (J. P. Corradi and R.B.D., unpublished data), but not of cdr13, a minor antigen (on Western blots of Purkinje extracts) that was also cloned with Yo antisera (76). Immunity to the cdr2 antigen in gynecologic tumors is associated with effective antitumor immunity; 45/52 (87%) of patients with the Yo antibody and gynecologic cancer have limited tumors (9).

The cdr2 gene (77, 78) encodes a protein of predicted M_r of 52 kDa that harbors an extended amphipathic helix-leucine zipper domain in its N-terminal one-third (79), and a unique sequence of unknown function in its C-terminal two-thirds, suggesting separate dimerization and functional domains. The disease epitope has been mapped using Yo antisera, and localizes to the N-terminal leucine zipper domain (79). The Yo antigen localizes to the cell soma and cytoplasmic fractions of neurons, suggesting that it may interact with other leucine zipper proteins there. Identification of dimerization partners for cdr2, as well as a complete study of the expression pattern of protein, may yield insight into its role in both neurons and gynecologic tumors.

Models of Disease Pathogenesis

The model for the pathogenesis of PNDs presented here (Fig. 1) has three essential features, which will be considered below: (*i*) onconeural antigens are normally expressed only in immune privileged sites and are immunogenic when ectopically expressed in tumors, (*ii*) antitumor immunity correlates with immunity to onconeural antigens, and (*iii*) antineuronal autoimmunity develops in a subset of patients with antitumor immunity.

Immune Privilege of Onconeural Antigens. Two components may be considered in establishing the immune privilege of onconeural antigens, one physical and one molecular. The blood-brain barrier is a physical barrier established, in part, by a specialized microvasculature and astrocytic foot processes that separates the CNS from the systemic circulation. This barrier can be broached by the immune system in disease (e.g., by cytokines such as IFN-γ and TNF-α), and may normally be "violated" by small numbers of immune cells that survey the CNS (80, 81). Thus, all individuals are likely to have some immune surveillance of the CNS by immune cells, and this mechanism is not likely to account for the strict immune privilege proposed for onconeural antigens. Moreover, some onconeural antigens (e.g., Hu antigens) are normally expressed in peripheral nervous system neurons (e.g., myenteric plexus neurons of the intestine) that lack a physical blood-brain barrier.

Molecular mechanisms of immune privilege are suggested by the observation that neurons do not normally appear to express major histocompatibility complex (MHC)-I molecules (82–84), or that testis, another immune privileged site, does express Fas (CD95) ligand (85). The absence of self-presentation molecules in neurons, or the induction of apoptosis of immune cells via Fas-like pathways (85, 86) provide mechanisms by which intracellular proteins (e.g., onconeural antigens) might entirely evade immune tolerance. Such a mechanism would be likely to be valid whether onconeural antigens are expressed in neurons of the gut, dorsal root ganglia, or CNS.

Tumor Immunity in PND. Immune privilege of onconeural antigens fails when the antigens are expressed in the tumors of PND patients. Two points broaden the scope of this observation. (*i*) While the neurologic complications found in the PNDs are rare (they complicate no more than 1 of 1000 cancer cases; see ref. 6), an unexpectedly large number of tumors are able to elicit immune responses to PND antigens. For example, 15% of small cell lung tumors are associated with low-titer antibodies to Hu antigens in the absence of neurologic disease, and these patients have a remarkably high percentage (>90%) of limited stage tumors (compared with small cell lung cancer patients who have no detectable Hu antibodies, 60–70% of whom have widely metastatic disease when diagnosed; see ref. 7). (*ii*) At least some PND antigens are expressed in a large percentage of tumors of a particular type. For example, the Hu antigens are expressed in all small cell lung tumors and most neuroblastomas (11, 87).

These observations suggest that ectopic expression of onconeural antigens may not be the sole determinant of their immunogenicity. Instead, in the course of evading immune surveillance, tumor cells may recapitulate some aspects of (molecular) immune privilege for certain onconeural antigens. Some support for this suggestion comes from evidence that a higher percentage of tumors associated with the Hu syndrome and antitumor immunity express MHC-I antigens than do tumors that are not associated with PND (11). Alternatively, host factors, such as specific MHC haplotypes, may act as determinants of whether an immune response is generated to onconeural antigens (or tumor cells), although no such correlation has been found.

The observed correlation between the development of detectable immunity to onconeural antigens and effective antitumor immunity does not suppose a cause and effect relationship between the two. Antitumor immunity, established independently from an immune response to onconeural antigens, might breach tumor immune privilege in such a way (e.g., by means of tumor cell apoptosis) that allows access to intracellular antigens and the secondary establishment of immunity to onconeural antigens. The proposal that immunogens such as RNA-binding proteins may be presented and targeted by the immune system in autoimmune disorders following apoptotic events (88, 89) suggests that similar mechanisms might lead to immunogenicity of some onconeural antigens— for example, the Nova or Hu RNA-binding proteins. It should be noted, however, that PND antibodies are typically found in isolation, not in conjunction with other autoantibodies, including antinuclear antibodies.

The relationship between tumor immunity and immunity to onconeural antigens appears to differ for the various PNDs. In the NMJ paraneoplastic syndromes, where autoantibodies appear to be sufficient to mediate neurologic disease, there is no evidence to suggest that the presence of antibodies is associated with antitumor immunity. This correlation has only been established in PNDs involving intracellular antigens (most notably the Hu and Yo antigens), syndromes in which antibodies have failed to passively transfer disease. These observations suggest that, for some PNDs, antitumor immunity and immunity to onconeural antigens could be causally linked, and should heighten interest in whether cellular immunity plays a pivotal role in these disorders.

Antineuronal Immunity in PND. With the exception of the PND antigens of the NMJ, the PND antigens discussed in this review have different cellular distributions—nerve terminal, nuclear, or somato-dendritic/cytoplasmic—but are all intracellular proteins (Table 2). This observation presents difficulty for the hypothesis that antibodies mediate paraneoplastic neurologic disease. Nevertheless, a pathogenic role for PND antibodies in neuronal dysfunction cannot be entirely excluded. Various reports have suggested that some neurons may selectively take up macromolecules, including antibodies, into their cytoplasm (90–93). As noted above, antineuronal antibodies are able to passively transfer disease to animals in MG and LEMS, providing a compelling precedent for disease pathogenesis in the remaining PNDs. Finally, there are relatively higher titers of antibody in the CSF than serum (IgG index >1; refs. 6, 94, and 95), suggesting that there is an active B-cell inflammatory response within the CSF compartment of PND patients. Depending on the nature of the protein, antibody inhibition of function could be reversible or lead to neuronal death, and there is evidence for both types of neurologic dysfunction in PNDs. A significant number of POMA patients have complete resolution of their neurologic symptoms (96), suggesting the presence of dysfunctional but intact neurons in some patients. However, it should be noted that the most typical pathologic finding in PND is neuronal degeneration (6).

Autoimmune antibodies are believed to frequently target functional protein domains (97), and this appears to be true for PND antibodies. For example, the cdr2 epitope is the leucine zipper dimerization domain of the protein (79), the Hu epitope includes two RNA-binding domains (58), and the Nova-1 epitope is the third KH RNA-binding domain (49). In the latter case, PND antibodies inhibit the functional ability of Nova-1 to bind to RNA *in vitro* (49).

An additional means of antibody toxicity specific to neurons has been suggested by the observation that antibodies, and not T cells, may be responsible for eradicating latent viral infections in neurons. Antibodies to alphaviral proteins presented at the surface of latently infected neurons appear to restrict the expression of those proteins and eradicate viral infection, while cytotoxic T lymphocytes capable of recognizing the same antigens presented via MHC-I do not (98). If intracellular antigens are presented on the surface of neurons, an antibody mediated signal within the neuron may thus be able to inhibit antigen expression and thereby effect neuronal function.

The role of killer cells in the pathogenesis of PND has not been thoroughly explored. Several features of the PNDs make killer cells such as CD8+ cytotoxic killer cells attractive candidates for mediators of disease. Most significantly, such cells have the potential to recognize intracellular onconeural antigens processed and presented via MHC-I molecules. An-

titumor immunity is thought to involve T-cell recognition of tumor antigens presented via MHC-I molecules (99, 100), in addition to other costimulatory signals; tumor immunity might set the stage for killer cell recognition of neurons. Moreover, the pathologic hallmark of PND is neuronal destruction (6, 101).

However, the lack of readily identifiable MHC expression on neurons (82) complicates the speculation that T cells are involved in the development of PNDs. While some studies have suggested that MHC molecules may be inducible in neurons *in vitro* (102), the significance of this observation *in vivo* is unclear. For example, transgenic mice made to express MHC-I molecules on neurons do not appear to undergo neuronal death following infection of neurons with lymphocytic choriomeningitis virus despite adoptive transfer of virus-reactive cytotoxic T lymphocytes (103). In summary, studies evaluating the pathogenesis of PND should consider the involvement of both classical and nonclassical T-cell types, including natural killer cells (which may recognize tumor or virally infected cells that fail to express MHC-I molecules; (104)), γ/δ cells, and CD4−/CD8− α/β cells that recognize antigens presented via MHC Ib and CD1 molecules (105, 106).

Concluding Remarks

PNDs are a diverse group of diseases. Cloning target antigens using PND antisera allows both the clinician and scientist to discriminate between disorders that are otherwise similar in their symptomatology and pathology. In this way, an expanding group of onconeural antigens has been identified that shares common features—neuron-specificity and intracellular localization—but that can also be classified into several distinct groups based on their function. An important unresolved issue is whether the three identified roles of onconeural proteins—tumor antigens, autoimmune antigens, and neuron-specific proteins—are related. For example, specific tumor types consistently express specific onconeural antigens (Table 1). This selectivity is likely to yield clues to the role of onconeural antigens in PND. Individual onconeural genes could provide functions co-opted by tumor cells (as in the case of signal transduction proteins)or they might act as particularly potent immunogens (as in the case of n-RBPs) or be exposed in particularly vulnerable ways to the immune system (nerve terminal vesicle-associated proteins). Similarly, onconeural genes might be activated in trans with an activity that is directly selected by tumor cells (e.g., coordinate transcriptional controls with a cellular oncogene) or their expression might relate to the cell of origin of the tumor (e.g., small cell lung cancer as a neuroectodermally derived tumor). Perhaps the PNDs are best viewed as a diverse group of neurologic disorders, in which mechanisms of disease may share some common features but have different pathophysiologies that are likely to relate to different families of antigens. The ability to classify the target antigens based on the sequence and function of the proteins will be of value in establishing the pathophysiology for individual disorders.

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1. Jaeckle, K., Graus, F., Houghton, A., Cordon-Cardo, C., Nielsen, S. L. & Posner, J. B. (1985) *Ann. Neurol.* **18,** 592–600.
2. Jaeckle, K. A., Houghton, A. N., Nielsen, S. L. & Posner, J. B. (1983) *Ann. Neurol.* **14,** 111.
3. Graus, F., Cordon-Cardo, C. & Posner, J. (1985) *Neurology* **35,** 538–543.
4. Anderson, N. E., Rosenblum, M. K. & Posner, J. B. (1988) *Ann. Neurol.* **24,** 559–567.
5. Budde-Steffen, C., Anderson, N., Rosenblum, M., Graus, F., Ford, D., Synek, B., Wray, S. & Posner, J. (1988) *Ann. Neurol.* **23,** 528–531.
6. Posner, J. B. & Furneaux, H. M. (1990) in *Immunologic Mechanisms in Neurologic and Psychiatric Disease*, ed. Waksman, B. H. (Raven, New York), p. 187–219.
7. Dalmau, J., Furneaux, H. M., Gralla, R. J., Kris, M. G. & Posner, J. B. (1990) *Ann. Neurol.* **27,** 544–552.
8. Dalmau, J., Furneaux, H. & Posner, J. (1991) *Proc. Am. Assoc. Cancer Res.* **32,** 272.
9. Peterson, K., Rosenblum, M. K., Kotanides, H. & Posner, J. B. (1992) *Neurology* **42,** 1931–1937.
10. Darnell, R. B. & DeAngelis, L. M. (1993) *Lancet* **341,** 21–22.
11. Dalmau, J., Graus, F., Cheung, N. V., Rosenblum, M. K., Ho, A., *et al.* (1995) *Cancer* **75,** 99–109.
12. Dalmau, J. & Posner, J. B. (1994) *Neurology* **44,** 2241–2246.
13. Zaheer, W., Friedland, M. L., Cooper, E. B., DoRosario, A., Burd, R. M., Gagliardi, J. & Torstenson, G. (1993) *Cancer Invest.* **11,** 306–309.
14. Dalmau, J., Graus, F., Rosenblum, M. K. & Posner, J. B. (1991 *Medicine* **71,** 59–72.
15. Blumenfeld, A. M., Recht, L. D., Chad, D. A., DeGirolami, U., Griffin, T. & Jaeckle, K. A. (1991) *Neurology* **41,** 1682–1685.
16. Fueyo, J., Gomez-Manzano, C., Pascual, J. & Pou, A. (1993) *Neurology* **43,** 236.
17. Cunningham, J., Graus, F., Anderson, N. & Posner, J. (1986) *Neurology* **36,** 1163–1168.
18. Thirkill, C. E., Fitzgerald, R. C., Sergott, A. M., Roth, N. K., Tyler, N. K. & Keltner, J. L. (1989) *N. Engl. J. Med.* **321,** 1589–1594.
19. Buckanovich, R., Posner, J. B. & Darnell, R. B. (1993) *Neuron* **11,** 657–672.
20. Luque, F., Furneaux, H., Ferziger, R., Rosenblum, M., Wray, S., *et al.* (1991) *Ann. Neurol.* **29,** 241–251.
21. Graus, F., Elkon, K. B., Cordon-Cardo, C. & Posner, J. B. (1986) *Am. J. Med.* **80,** 45–52.
22. Kornstein, M. J., Asher, O. & Fuchs, S. (1995) *Am. J. Pathol.* **146,** 1320–1324.
23. Gattenlohner, S., Brabletz, T., Schultz, A., Marx, A., Muller-Hermelink, H. K. & Kirchner, T. (1994) *Thymus* **23,** 103–113.
24. Roberts, A., Perera, S., Lang, B., Vincent, A. & Newsom-Davis, J. (1985) *Nature (London)* **317,** 737–739.
25. Lennon, V. A., Kryzer, T. J., Griesmann, G. E., O'Suilleabhain, P. E., Windebank, A. J., Woppmann, A., Miljanich, G. P. & Lambert, E. H. (1995) *N. Engl. J. Med.* **332,** 1467–1474.
26. Fukunaga, H., Engel, A. G., Lang, B., Newsom-Davis, J. & Vincent, A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7636–7640.
27. Motomura, M., Johnston, I., Lang, B., Vincent, A. & Newsom-Davis, J. (1995) *J. Neurol. Neurosurg. Pyschiatry* **58,** 85–87.
28. Tzartos, S. J. & Lindstrom, J. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 755–759.
29. Beroukhim, R. & Unwin, N. (1995) *Neuron* **15,** 323–331.
30. Toyka, K. V., Drachman, D. B., Pestronk, A. & Kao, I. (1975) *Science* **190,** 397–399.
31. Fukuoka, T., Engel, A. G., Lang, B., Newsom-Davis, J., Prior, C. & Wray, D. W. (1987) *Ann. Neurol.* **22,** 193–199.
32. Lang, B., Newsom-Davis, J., Wray, D. & Vincent, A. (1981) *Lancet* **ii,** 224–226.
33. Newsom-Davis, J., Vincent, A., Wilson, S. G., Ward, C. D., Pinching, A. J. & Hawkney, C. (1978) *N. Engl. J. Med.* **298,** 456.
34. Newsom-Davis, J. & Murray, N. M. F. (1984) *Neurology* **34,** 480–485.
35. Smitt, P. A. E. S., Manley, G. T. & Posner, J. B. (1995) *Neurology* **45,** 1873–1878.
36. Sakai, K., Gofuku, M., Kitagawa, Y., Ogasawara, T. & Hirose, G. (1995) *J. Neuroimmunol.* **60,** 135–141.
37. Solimena, M., Folli, F., Denis-Donini, S., Comi, G., Pozza, G., DeCamilli, P. & Vicari, A. (1988) *N. Engl. J. Med.* **318,** 1012–1020.
38. Christgau, S., Aanstoot, H.-J., Schierbeck, H., Begley, K., Tulin, S., Hejnaes, K. & Baekkeskov, S. (1992) *J. Cell Biol.* **118,** 309–320.
39. Elferink, L. A., Peterson, M. R. & Scheller, R. H. (1993) *Cell* **72,** 153–159.
40. DeCamilli, P., Thomas, A., Cofiell, R., Folli, F., Lichte, B., *et al.* (1993) *J. Exp. Med.* **178,** 2219–2223.

41. David, C., Mcpherson, P. S., Mundigl, O. & Decamilli, P. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 331–335.
42. Wang, L. H., Südhof, T. C. & Anderson, R. G. W. (1995) *J. Biol. Chem.* **270,** 10079–10083.
43. Newman, L. S., McKeever, M. O., Okano, H. J. & Darnell, R. B. (1995) *Cell* **82,** 773–783.
44. Darnell, R. B., Furneaux, H. M. & Posner, J. R. (1991) *J. Neurosci.* **11,** 1224–1230.
45. Martin-Moutot, N., el Far, O., Leveque, C., David, P., Marqueze, B. *et al.* (1993) *J. Physiol. (London)* **87,** 37–41.
46. Leveque, C., Hoshino, T., David, P., Shoji-Kasai, Y., Leys, K., *et al.* (1992) *Proc. Natl. Acad. Sci. USA* **89,** 3625–3629.
47. Rosenfeld, M. R., Wong, E., Dalmau, J., Manley, G., Posner, J. B., Sher, E. & Furneaux, H. M. (1993) *Ann. Neurol.* **33,** 113–120.
48. Burd, C. & Dreyfuss, G. (1994) *Science* **265,** 615–621.
49. Buckanovich, R. J., Yang, Y. Y. & Darnell, R. B. (1996) *J. Neurosci.* **16,** 1114–1122.
50. Casado, J. L., Gil-Peralta, A., Graus, F., Arenas, C., Lopez, J. M. & Alberca, R. (1994) *Neurology* **44,** 1521–1522.
51. Graus, F., Rowe, G., Fueyo, J., Darnell, R. B. & Dalmau, J. (1993) *Neurosci. Lett.* **150,** 212–214.
52. Siomi, H., Siomi, M., Nussbaum, R. & Dreyfuss, G. (1993) *Cell* **74,** 291–298.
53. Siebel, C. W., Admon, A. & Rio, D. C. (1995) *Genes Dev.* **9,** 269–283.
54. Engebrecht, J. A., Voelkel-Meiman, K. & Roeder, G. S. (1991) *Cell* **66,** 1257–1268.
55. Szabo, A., Dalmau, J., Manley, G., Rosenfeld, M., Wong, E., Henson, J., Posner, J. B. & Furneaux, H. M. (1991) *Cell* **67,** 325–333.
56. Robinow, S., Campos, A., Yao, K. & White, K. (1988) *Science* **242,** 1570–1572.
57. Yao, K.-M., Samson, M.-L., Reeves, R. & White, K. (1993) *J. Neurobiol.* **24,** 723–739.
58. Manley, G. T., Smitt, P. S., Dalmau, J. & Posner, J. B. (1995) *Ann. Neurol.* **38,** 102–110.
59. Posner, J. B. (1995) *Perspect. Biol. Med.* **38,** 167–181.
60. King, P., Levine, T., Fremeau, R. & Keene, J. (1994) *J. Neurosci.* **14,** 1943–1952.
61. Levine, T. D., Gao, F., King, P. H., Andrews, L. G. & Keene, J. D. (1993) *Mol. Cell. Biol.* **13,** 3494–3504.
62. Sakai, K., Gofuku, M., Kitagawa, Y., Ogasawara, T., Hirose, G., *et al.* (1993) *Biochem. Biophys. Res. Commun.* **199,** 1200–1208.
63. Zubiaga, A. M., Belasco, J. G. & Greenberg, M. E. (1995) *Mol. Cell. Biol.* **15,** 2219–2230.
64. Lagnado, C. A., Brown, C. Y. & Goodall, G. J. (1994) *Mol. Cell. Biol.* **14,** 7984–7995.
65. McMullough, A. J. & Schuler, M. A. (1993) *Mol. Cell. Biol.* **13,** 7689–7697.
66. Bandzuilis, R., Swanson, M. & Dreyfuss, G. (1989) *Genes Dev.* **3,** 431–437.
67. Baker, B. (1989) *Nature (London)* **340,** 521–524.
68. Valcarcel, J., Singh, R., Zamore, P. & Green, M. (1993) *Nature (London)* **362,** 171–175.
69. Amara, S., Jonas, V., Rosenfeld, M., Ong, E. & Evans, R. (1982) *Nature (London)* **298,** 240–244.
70. Rosenfeld, M. G., Mermod, J. J., Amara, S. G., Swanson, L. W., Sawchenko, P. E., Rivier, J., Vale, W. W. & Evans, R. M. (1983) *Nature (London)* **304,** 129–135.
71. Gao, F. B., Carson, C. C., Levine, T. & Keene, J. D. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 11207–11211.
72. Thirkill, C. E., Tait, R. C., Tyler, N. K., Roth, A. M. & Keltner, J. L. (1992) *Invest. Ophthalmol. Vis. Sci.* **33,** 2768–2772.
73. Polans, A. S., Buczylko, J., Crabb, J. & Palczewski, K. (1991) *J. Cell. Biol.* **112,** 981–989.
74. Lem, J., Flannery, J. G., Li, T., Applebury, M. L., Farber, D. B. & Simon, M. I. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4422–4426.
75. Smith, D. P., Ranganathan, R., Hardy, R. W., Marx, J., Tsuchida, T. & Zuker, C. S. (1991) *Science* **254,** 1478–1484.
76. Dropcho, E. J., Furneaux, H., Tseng-Chen, Y., Old, L. J. & Posner, J. B. (1988) *Ann. Neurol.* **24,** 121–122.
77. Fathallah-Shaykh, H., Wolf, S., Wong, E., Posner, J. & Furneaux, H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3451–3454.
78. Sakai, K., Mitchell, D., Tsukamoto, T. & Steinman, L. (1990) *Ann. Neurol.* **28,** 692–698.
79. Sakai, K., Ogasawara, T., Hirose, G., Jaeckle, K. A. & Greenlee, J. E. (1993) *Ann. Neurol.* **33,** 373–380.
80. Couraud, P. O. (1994) *J. Leukocyte Biol.* **56,** 407–415.
81. Williams, K. C. & Hickey, W. F. (1995) *Curr. Top. Microbiol. Immunol.* **202,** 221–246.
82. Lampson, L. (1987) *Trends Neurosci.* **10,** 211–216.
83. Lampson, L. A., Grabowska, A. & Whelan, J. P. (1994) *Prog. Brain Res.* **103,** 307–317.
84. Wucherpfennig, K. W. (1994) *Clin. Immunol. Immunopathol.* **72,** 293–306.
85. Bellgrau, D., Gold, D., Selawry, H., Moore, J., Franzusoff, A. & Duke, R. C. (1995) *Nature (London)* **377,** 630–632.
86. Griffith, T. S., Brunner, T., Fletcher, S. M., Green, D. R. & Ferguson, T. A. (1995) *Science* **270,** 1189–1192.
87. Dalmau, J., Furneaux, H. M., Cordon-Cardo, C. & Posner, J. B. (1992) *Am. J. Pathol.* **141,** 881–886.
88. Casciola-Rosen, L. A., Anhalt, G. & Rosen, A. (1994) *J. Exp. Med.* **179,** 1317–1330.
89. Tan, E. (1994) *J. Exp. Med.* **179,** 1083–1086.
90. Borges, L. F., Elliott, P. J., Gill, R., Iversen, S. D. & Iversen, L. L. (1985) *Science* **228,** 346–348.
91. Fabian, R. H. & Petroff, G. (1987) *Neurology* **37,** 1780–1784.
92. Graus, F., Illa, I., Agusti, M., Ribalta, T., Cruz-Sanchez, F. & Juarez, C. (1991) *J. Neurol. Sci.* **106,** 82–87.
93. Greenlee, J. E., Parks, T. N. & Jaeckle, K. A. (1993) *Neurology* **43,** 2049–2054.
94. Graus, F., Sequrado, O. G. & Tolaso, E. (1988) *Acta Neurol. Scand.* **78,** 210–213.
95. Furneaux, H. L., Reich, L. & Posner, J. P. (1990) *Neurology* **40,** 1085–1091.
96. Darnell, R. B. (1994) in *Current Diagnosis in Neurology*, ed. Feldmann, E. (Mosby, Philadelphia), pp. 137–141.
97. Tan, E. (1991) *Cell* **67,** 841–842.
98. Levine, B., Hardwick, J. M., Trapp, B. D., Crawford, T. O., Hollinger, R. C. & Griffin, D. E. (1991) *Science* **254,** 856–860.
99. van der Bruggen, P., Traversari, C., Chomez, P., Lurquin, C., de Plaen, E., Van den Eynde, B., Knuth, A. & Boon, T. (1991) *Science* **254,** 1643–1647.
100. Chen, L., Ashe, S., Brady, W. A., Hellström, I., Ledbetter, J. A., McGowan, P. & Linsley, P. S. (1992) *Cell* **71,** 1093–1102.
101. Hormigo, A., Dalmau, J., Rosenblum, M. K., River, M. E. & Posner, J. B. (1994) *Ann. Neurol.* **36,** 896–902.
102. Neumann, H., Cavalie, A., Jenne, D. E. & Wekerle, H. (1995) *Science* **269,** 549–552.
103. Rall, G. F., Mucke, L. & Oldstone, M. B. A. (1995) *J. Exp. Med.* **182,** 1201–1212.
104. Gumperz, J. E. & Parham, P. (1995) *Nature (London)* **378,** 245–248.
105. Porcelli, S. A. & Modlin, R. L. (1995) *J. Immunol.* **155,** 3709–3710.
106. Bendelac, A. (1995) *Science* **269,** 185–186.
107. Simpson, F., Bright, N. A., West, M. A., Newman, L. S., Darnell, R. B. & Robinson, M. S. (1996) *J. Cell Biol.*, in press.