Neurologists have been fascinated by the paraneoplastic neurologic disorders (PNDs) for over a century. In the past 20 years, PNDs have emerged as prime examples of the way in which modern molecular biology can be used to transform esoteric medicine into widely applicable scientific and clinical insights.

Patients with PNDs typically exhibit rapid-onset, severe, and focal neurologic disease on initial examination. Magnetic resonance imaging findings are usually normal, and medical history and physical examinations yield no obvious cause for the disorders. An occult malignancy is usually discovered as patients are followed up or when a PND diagnosis is suspected. It is believed that such malignancies underlie the neurologic degeneration in PNDs. More specifically, breast, ovarian, or small cell lung cancer (or sometimes other tumor types [Table]) are found to be present, often only in a lymph node or as a small focus of tumor. A critical link between these tumors and the neurologic degeneration found at a distance from the tumor site—the hallmark of PND—was established by Jerome Posner and colleagues in the early 1980s.1,2 These investigators discovered that patients with PND harbor high-titer antibodies that recognize antigens expressed in their tumors and in regions of the brain undergoing degeneration. It has subsequently been shown that the expression of these antigens is normally entirely restricted to neurons, from early development onward. This restriction is believed to underlie the intense immune response elicited by the antigens when they are ectopically expressed in cancer cells.3,4

The discovery of PND-associated antibodies formed the basis for both basic and clinical advances. Our laboratory and others helped pioneer the use of PND antiserum samples (Table). Research on the paraneoplastic opsoclonus-myoclonus ataxia (POMA) antigen, Nova, illustrates the way in which these cDNAs have been used to develop new insight into neurobiology.5 Nova was identified using antiserum samples from a patient with POMA to screen a cerebellar cDNA expression library.6 The cDNA encodes a protein harboring 3 KH-type RNA binding motifs, and the Nova gene is expressed exclusively in a subset of central nervous system neurons (primarily brainstem and ventral spinal cord neurons) at all developmental stages (E 8.5 through adult) examined. The function of the Nova protein was discovered using an approach that began with a biochemical method termed RNA selection, used to determine the specific RNA sequences to which the Nova protein binds. These experiments,7 subsequently coupled with x-ray crystallography,8 revealed that the Nova KH domains
fold to form Watson-Crick hydrogen bond donor-acceptors, in the manner that a second strand of DNA would, to repeat UCAU RNA sequences.

With this information in hand, database searches and additional biochemical experiments identified a set of candidate neuronal genes encoding RNAs that express such UCAU repeat elements. Validating the interaction of Nova with these target RNAs in the brain was possible by generating Nova knock-out mice, in which the Nova gene is no longer expressed. When the candidate RNA targets were analyzed, their metabolism was found to be dysregulated in wild-type vs KO littermates. New methods have now enabled the identification and validation of large numbers of Nova RNA targets.

These experiments revealed that Nova regulates alternative splicing in neurons. Splicing is the process by which a single pre–messenger RNA (mRNA), freshly transcribed, has its final coding and regulatory fragments (exons) linked together, cutting out intervening RNA elements (introns). Nova was the first tissue-specific (in this case, neuron-specific) mammalian protein capable of regulating whether optional exons—encoding additional genetic information—were included. Such regulation of alternative splicing may be particularly important in generating complexity in the brain. If a single transcript harbors 10 alternatively spliced exons, 2 or 1024 different proteins can be generated from a single gene, making those of us with perhaps as few as 30000 genes feel a bit more complicated.

The Nova antigens represent a neuron-specific system that regulates gene expression at the level of RNA metabolism, yet the function of such a system may go beyond the regulation of Nova RNA regulated by Nova, the processed glycine receptor

PND ANTIGENS AND HUMAN TUMOR IMMUNITY

Clinically, the discovery of PND antibodies provided a means for diagnosing the disorders. Particularly instrumental was the cloning of cDNAs that encode PND antigens, which has provided recombinant fusion proteins and, thereby, the establishment of specific assays. Not surprisingly, this has allowed a refined understanding of the clinical history of the PNDs that was not previously possible.

Moreover, the identification of cDNAs that encode PND antigens has allowed progress to be made in our understanding of the pathophysiologic mechanisms behind the disorders. The immune systems of patients with PNDs respond effectively to the tumors of these patients. Hence, they provide a window into an otherwise invisible clinical phenomenon—occult cancer under immune attack. We have recently reviewed the evidence for tumor immunity in PND. The availability of cDNAs that encode PND antigens has led to new insights into the important role of the immune system in PNDs. The original idea that the PND autoantibodies, like the autoantibodies described in myasthenia gravis or Lambert-Eaton myasthenic syndrome, might be sufficient to transfer disease came under question following a lack of experimental support and the sequence of the cDNAs themselves. The PND cDNAs, aside from those associated with disease at the neuromuscular junction, predicted antigens that were only present inside cells.

We hypothesized that a cellular immune response—similar perhaps to immune responses that are able to detect intracellular viruses—might play a role in PND pathogenesis. Using PND cDNAs to predict peptide motifs capable of binding major histocompatibility complex I molecules, we established that all of our patients with paraneoplastic cerebellar degeneration and occult breast or ovarian tumors also harbored PND antigen-specific killer T cells in their blood. These observations established a first plausible pathophysiologic link between the immune response to intracellular antigens and the clinical phenomen-
phenon of PND. Subsequent studies suggest that similar phenomena may underlie other PNDs, although rigorous proof remains to be demonstrated.1 Thus, it seems that PND antibodies are correct markers of specific disease phenomena in the same way that antiviral antibodies mark a patient as having had a specific infection; however, T cells mediate the actual cellular destruction of PND tumors.

The ability to understand the elements of successful tumor immunity has led us to explore the means by which the cellular immune response is initially triggered in PNDs. At issue has been the fact that T cells initially meet antigens in lymph nodes, where they encounter antigens presented by antigen-presenting cells (ie, dendritic cells) and are thereby able to be activated. Classical teaching dictates that cells present their own intracellular proteins, captured from within as they are being turned over in lysosomes, as peptide antigens on the extracellular surface of major histocompatibility complex I molecules. In this way, intracellular viruses or parasites can be exposed to the cellular arm of the immune system. However, for a PND immune response to become initiated, PND-specific T cells would have to become activated by dendritic cells expressing these neuron-specific antigens. We had developed a substantial amount of data demonstrating that the only normal cells in the body that express PND antigens are neurons and were thus faced with a paradox as to how a T-cell response could be initiated.

A solution to this problem, as well as to the more general issue of how dendritic cells trigger immune responses to antigens that are not expressed inside dendritic cells was suggested by the work of Casciola-Rosen and colleagues.16 These investigators in studying systemic lupus erythematosus discovered that apoptotic cells are able to package their intracellular antigens into small membranous bodies. We speculated that such apoptotic bodies provided a physical means by which antigens could be transferred from dying cells to the immune system for antigen presentation.17 When this idea was tested in both a viral system and in PND patients, it was found that apoptotic tumor cells15 or apoptotic virally infected cells17 provided a potent means for transferring antigens to dendritic cells for the activation of an antigen-specific immune response.

These pathophysiologic observations have great clinical importance. Our working model for successful tumor immunity in PND is that it is triggered by apoptotic tumor cells that transfer antigens to dendritic cells, which then migrate to lymph nodes to activate PND antigen-specific killer T cells. Since such cells kill targets by inducing apoptotic death, a potential positive feedback may be established that allows for additional, less immunogenic tumor antigens to be presented via additional apoptotic material to activate additional killer T cells (the phenomenon of epitope spread). In this way, a clinically relevant tumor immune response may be generated.

Moreover, one may envision recapitulating this process for the treatment of cancer in the general population of cancer patients. Tumor cells obtained from patients or tissue culture cells can be made apoptotic and cocultured with autologous antigen-presenting cells from cancer patients. We have demonstrated that dendritic cells obtained from patients with prostate cancer are capable of presenting a model antigen from apoptotic prostate tumor cells for the activation of antigen-specific T cells.19 Furthermore, this approach to mimicking the triggering of successful tumor immune responses in patients with PND is currently being tested in a phase 1 clinical study by our group. The bedside to bench and back again approach to PNDs is allowing the secrets of these rare disorders to be revealed and, hence, to make general contributions to science and medicine.

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REFERENCES