Editorial

NEURONAL DEGENERATION IN MALIGNANCY: AN UNDERSTANDING FOR IMMUNE PATHWAYS

Cancer is a mysterious disease which is known to have existed since ancient times. For the last few decades there has not been a great breakthrough in the cancer treatment. But in the last few years there has been a trend toward understanding and then managing this fatal disease by understanding biological pathways. Even with this a great success is still awaited. Tumor immunology and immunological surveillance has been explored extensively. Paraneoplastic diseases have provided a good ground to study these mechanisms.

Paraneoplastic neurological degenerations (PNDs) are a diverse group of human neurodegenerative diseases that are associated with cancer and with antitumor immunity. These are of many different types and one of them is called paraneoplastic cerebellar degeneration (PCD). In this type the patient initially experience loss of motor control. Several other types of PNDs can have symptoms like memory loss, sensory loss, cerebellar dysfunction, motor dysfunction or blindness. Each of these disorders is associated with characteristic tumor types, most commonly breast, and ovarian or small cell lung cancer.

It is believed that the link between the neurological degeneration and cancer in PND is an immunological one. Specifically, an immune response to neuronal proteins that are produced by the tumor cells are recognized and suppressed. In patients with PND, this phenomenon becomes clinically evident, leading to autoimmunity with neurological symptoms. Although the disorders are rare, affecting perhaps 1 in 10,000 patients with cancer, they offer the possibility to study immunity that is directed against the defined set of tumor antigens.

PNDs are initiated when tumor cells produce proteins that are normally present in the neurons. Neurons are restricted from immune surveillance both by a physical blood brain barrier and by variable and low levels of major histocompatibility complex molecules. Expression of PND associated antigens by peripheral tumors has the potential to trigger antitumor immune responses. This only happens after a confluence of events. First of all tumor cells undergo apoptosis. These apoptotic cells can be engulfed by tissue dendritic cells (DCs); these cells must compete with other phagocytic cells for example, macrophages, which will also engulf apoptotic cell fragments, but are not competent to initiate T cell responses. DCs phagocytose apoptotic cells and then go to the lymph node and mature, which involves the down regulation of their phagocytic capacity and the up regulation of their ability to activate T cells. Within the lymph node, DCs present antigens from the phagocytosed tumor cell to both CD4+ and CD8+ T cells thereby increasing the response. Activated CD8+ T cells return to the tumor and further induce apoptotic cell death. This process also spread the immune response to less reactive T cells, thereby augmenting the autoimmunity. Subsequently immune recognition of neurons that normally express the target PND antigens leads to the clinical signs and symptoms of neuronal degeneration.

It was proposed long before that these immune mechanisms could act as a natural defense against tumor cells. CD8+ T cells or cytotoxic T cells express receptors (TCR) that recognize the antigen peptides on the surface of any host cell, in association with major histocompatibility complex class I (MHC I). T cell receptors bind to a triad of three proteins on the surface of target cells. These are MHC I molecules, β_2 microglobulin and short peptides that sit on the surface of the MHC I molecule. MHC I molecules bind peptide fragments derived from proteolytically degraded antigens that are endogenously synthesized by a cell. These peptides can be derived from any type of intracellular

proteins, whether nuclear or membrane associated. Cells can present peptide antigens that they acquire through infection with parasites or viruses, as well as through neoplastic transformation. Small antigenic peptides are transported into the endoplasmic reticulum (ER), where they associate with nascent MHC I molecules before being routed through the ER and displayed on the surface.

It is seen for many years that patients with PNDs have a more indolent course than comparable populations of patients with non PND associated cancer. However, it is known that majority of PND patients do not have effective antitumor immune responses that can lead to tumor degeneration. In fact, in over 90% of individuals with PND, tumors can be identified. This shows that the antitumor immunity that is associated with PND is incomplete.

Evidence of effective antitumor immunity in PND has been most compelling in studies of patients at the early stages of their illness. PND patients are typically unaware that they have cancer when they become ill, as the diagnosis of the neurological disorder precedes the diagnosis of the cancer in at least two-thirds of cases. However when found, PND associated tumors are limited in their growth. By contrast, over 60% of small cell lung cancers that are not associated with PNDs have already progressed to metastatic disease at the time of diagnosis. Similarly, in over 60% of patients with PCD, neurological symptoms preceded the cancer diagnosis and 82% had non metastatic tumors. It was seen that about 50% of unselected patients with breast cancer and 25% of patients with ovarian cancer present with limited stage disease. In another report, only 33% patients with PCD associated ovarian cancer and 8% with PCD associated breast cancer had metastatic disease. Whereas 60% in the general population of patients with ovarian cancer has metastatic disease. As a group, patients with PND have an improved cancer prognosis relative to patients with histologically identical tumors that are not associated with PND. Such observations have been reported for the majority of the PNDs.

Overall, in approximately 5-10% of patients with PCD no tumor is ever found, potentially reflecting effective tumor suppression. So, the onset of neurodegenerative symptoms and immune response seems to correlate with tumor suppression in many patients with PNDs. One hallmark of PCD associated gynecological tumors is the presence of inflammatory plasma cells and T cells.

In the 1980s a link between neurodegeneration and cancer in patients with PND was made by describing high titers of antibodies in the serum and cerebral spinal fluid of patients with PND. These antibodies recognized unique proteins that were present in regions of the brain undergoing degeneration and similarly recognized antigens of the same apparent molecular weight in the tumors that were obtained from these patients. These observations led to an initial model for PND in which expression of neuronal proteins in a tumor triggers an antitumor antibody response that subsequently crosses the blood brain barrier and induces autoimmune neurodegeneration.

It was initially believed that PND related antigens were only expressed in rare tumors and that their expression was necessary and sufficient to induce autoimmunity. However, these observations implied that expression of the PND antigen was necessary and sufficient for the development of this syndrome. Further evaluation led to the observation that at least some PND associated antigens are commonly formed by specific tumor types. It is clear that the infrequency of the PND syndromes cannot be attributed to the infrequency of PND associated antigen expression. So, if PND associated antigens are expressed by tumors in patients that do not develop neurodegenerative disorders do these patients develop antitumor immunity? 15% of patients with small cell lung cancer without PND were found to have low titers of antibody. Importantly, antibody production

was correlated with limited stage disease, complete responses to chemotherapy and with survival.

A significant population of patients with cancer naturally produces an antitumor immune response. Moreover, the frequency with which these tumor immune responses were observed indicates two points. Most patients with tumors that form potentially immunogenic antigens fail to mount an antitumor immune response. A better understanding of this failure could yield insight into mechanisms by which tumors evade immune surveillance, or by which T cell tolerance towards the tumor arises. Secondly, a significant percentage of patients are able to mount antitumor immune responses that are at least partially effective but do not develop PND. These patients fare better than individuals who do not develop an antitumor immune response. These observations contradict the idea that antitumor immune responses are extremely rare phenomenon, or that they are always associated with autoimmunity. Further study of the different cancer populations that develop PND immune responses with or without neurological disease could yield important insight into both antitumor immunity and what is required to tip the balance toward autoimmunity. Studies of peripheral blood from patients with PND clearly documented the presence of PND antigen specific CD8+/cytotoxic T lymphocytes (CTLs). These studies indicate that T cell responses are an important aspect of antitumor immunity in PND.

A key observation that shed some light on the mechanism by which PND antigens are transferred to DCs came from an observation that was made in the study of the autoimmune disease systemic lupus erythematosis (SLE). Patients with SLE frequently experience flares in their disease after exposure to sunlight. It was experimented in vitro and was found that ultraviolet irradiation of keratinocytes causes them to undergo apoptosis, allowing the lupus antigen to be pushed out of the dying cell. From these studies, it was clear that antigens derived from apoptotic cells could be taken in by DCs, leading to activation of antigen specific T cells. Apoptotic tumor cells were found to be an extremely effective source of antigen for maturing DCs, which were able to activate potent PND antigen specific killer T cells from the lymphocytes of patients with PCD.

DCs exist in the body as an immature cell responsible for capturing antigens, including fragments of apoptotic cells. After the DC has captured apoptotic tumor cells, it must gain access to the draining lymph organ, the site of T cell activation. Following capture of apoptotic tumor cells, DCs mature and up regulate receptor that is essential for accessing the T cell rich zones of the lymph node. Within these zones, DCs engage T cells to initiate immune responses. This ability to move out of peripheral tissue with captured antigen and enter the afferent lymph is unique to DCs.

The possible outcomes of this whole affair are either T cell activation or T cell tolerance. It was found in one experiment that DCs were found to present antigens to CD8+ T cells in the absence of CD4+ helper T cells and to trigger a tolerance pathway, in which antigen specific CD8+ T cells undergo a couple of cell divisions and then die an apoptotic cell death. Conversely, the presence of CD4+ and CD8+ T cells leads to T cell activation. From in vivo studies, it was clear that the presence versus the absence of antigen reactive CD4+ T cells serves as a crucial determinant by which CD8+ T cell activation or tolerance is determined. Although it remains somewhat controversial, it is believed that T cell tolerance and activation, including tumor mediated T cell responses. Both occur in the lymph node and are mediated by DCs that have up regulated receptor.

Therefore, in different patients who have PND antigen presenting tumors, several variables can now be considered to be important determinants of whether patients

respond or fail to respond. These variables include the extent of tumor cell apoptosis, the penetration of DCs into the tumor tissue and, importantly, the activities of CD4+ helper T cells in the lymph node, as well as individual variation in the CD8+ and CD4+ T cell of the patients.

The study of PNDs has yielded a wealth of new information about human tumor immunity and a more complex model for the disorders themselves. Importantly, these studies have indicated that the antitumor immune response can effectively suppress tumor growth and is associated with the presence of PND antigen specific CTLs. The T cell antitumor response might be activated by DCs that have engulfed apoptotic tumor cells. So activated CTLs can kill targets by inducing apoptotic cell death, which in return allows the amplification of the T cell antitumor immune response.

Apoptotic cancer cells could be presented to dendritic cells in vitro. These cells could be given back to patients, allowing stimulation of both CD8+ and CD4+ T cells. The coordinated activation of both cell types is likely to limit the development of autoimmune responses. However the patients that develop antitumor CD8+ T cells could also fail to initiate an effective antitumor response if their CD4+ T cell is limited or somehow inhibited. In this context, agents that activate a CTL response by bypassing the need for CD4+ T can help this process. These adjuvant such as TNF super family members, which provide CD4+ signals to dendritic cells, could be incorporated into tumor immunotherapy that use apoptotic tumor cell presentation. It will be interesting to determine whether it is possible to apply the potency of apoptotic tumor cells, as a means of activating tumor-specific T cells, to cancer therapy in general.

The study of patients with PND has provided proof in principle that the tumor surveillance hypothesis is really important in humans. This has yielded important clinically relevant and basic insights into the mechanisms by which antitumor immunity can be triggered. PND antigens are more widely expressed in tumor cells than was originally thought, so further studies are required to increase our understanding of how tumor immunity can be successfully triggered, and why it doesn't happen so frequently.

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Dr Tariq Parvez
Consultant Oncologist
King Fahad Hospital
Al Madina Al Munawra
Kingdom of Saudi Arabia

Dr Babar Parvez
PSCP Lahore, Pakistan