[CANCER RESEARCH 56, 4278–4281, September 15, 1996]

Meeting Report

Psychoneuroimmunology and Cancer: Fifteenth Sapporo Cancer Seminar[1]

The relatively recent field of multidisciplinary scientific inquiry known as PNI[2] was first described comprehensively in 1981. This field has moved rapidly beyond the initial concentration on establishing the existence of interactions across the CNS to include mental as well as neurological phenomena and the endocrine, immune, and autonomic systems.

The 15th Sapporo Cancer Seminar was held to establish an understanding of how and to what extent PNI is relevant to oncology. Cancer is a disease that involves highly diversified processes, ranging from mechanisms that underlie the generation of tumor cells to the interaction between these cells and the host. The multifactorial aspects of neoplasia were reflected in the variety of topics covered in this meeting. Some of these topics were related to the functional aspects of transforming genes, the molecular, genetic, and cellular processes involved in carcinogenesis, and how tumor cells escape immunosurveillance. However, as pointed out by H. Kobayashi (The Sapporo Cancer Seminar Foundation, Sapporo, Japan), a major objective of this meeting was to focus on concepts as well as on findings of how immune, tumoral, and other factors can interact with neuroendocrine mechanisms, and conversely, how processes acting at the level of the CNS, *e.g.*, stress, can affect the activity of immune and neoplastic cells. As mentioned by F. Sendo (Yamagata University, Yamagata, Japan), another objective of the meeting was to discuss how multiple mechanisms are integrated at the CNS level, how they affect the host response to a tumor, and how they help determine the consequences of this response on tumor progression.

The seminar opened with introductory remarks by R. Herberman (Pittsburgh Cancer Institute, University of Pittsburgh, Pittsburgh, PA). He first emphasized the need for a truly multidisciplinary collaboration among researchers of different specialities, from psychologists and psychiatrists to neurobiologists, endocrinologists, immunologists, and cancer biologists. Toward this end, he added that it is important to try to overcome the difficulties arising from differences in the terminology used by the various disciplines. He also pointed out some critical issues worth considering: (*a*) one major issue is the influence of psychological factors, particularly the reaction to stress, on the development and progression of cancer. However, it should be emphasized that although psychological factors may affect the course of cancer growth and/or regulate the host's defenses against cancer, they are not responsible for the onset of carcinogenesis itself; (*b*) there are likely to be a number of steps connecting CNS activities and neoplastic processes, with the neuroendocrine and immune systems being the intermediary links; and (*c*) the ultimate goal in this type of research is to better understand the impact of each of these events on the cancer patient so that we can use these observations as a starting point for working out the clinical applications.

Received 1/22/96; accepted 7/10/96.

[1] This seminar was held July 5–8, 1995, and was organized by Drs. F. Sendo (Chairman), I. Azuma, H. O. Besedovsky, R. B. Herberman, N. Ohsawa, C. D. Spielberger, A. Uchida, and K. S. Zänker. Support was provided by the Japan Society for the Promotion of Science and the Uehara Foundation.

[2] The abbreviations used are: PNI, psychoneuroimmunology; CNS, central nervous system; HPA, hypothalamic-pituitary-adrenal; NGF, nerve growth factor; NK, natural killer; QOL, quality of life; BRM, biological response modifier; ATK, autologous tumor killing; IL, interleukin; ACTH, adrenocorticotropic hormone; CMV, cytomegalovirus.

Basic Concepts in Neuro-Endocrine-Immune Interactions

It is well established that many tumors are hormone dependent and that various types of immune cells are involved in immunosurveillance. Moreover, there is now clear evidence that immune and neuroendocrine mechanisms are closely interrelated. This topic was introduced by H. Besedovsky (Institute for Normal and Pathological Physiology, Philipps University, Marburg, Germany), who discussed various findings concerning these interactions. Immune cells are able to express various receptors for hormones, neurotransmitters, and neuropeptides, which are known to affect the immune response. Conversely, immune cells produce cytokines capable of affecting neuroendocrine functions. These interactions are reflected by functional changes occurring at the level of the CNS and peripheral nervous system and by endocrine activity during the course of an immune response. As an example of these types of interactions, H. Besedovsky discussed the activation of the HPA axis following administration of innocuous antigens as well as activation of the axis during the course of infective, inflammatory, autoimmune, and neoplastic processes. In most of these cases, stimulation of the HPA axis is mediated by cytokines released during activation of immune cells, such as IL-1, IL-6, and tumor necrosis factor. Taken together with the known effect of glucocorticoids on cytokine production and on different immune cell types, this demonstrates the existence of an integrated feedback circuit. He stressed the relevance of this circuit in immunoregulation and in host defense during induced sepsis, viral infection, autoimmune processes, and cancer.

T. Hirano (Osaka University, Osaka, Japan) showed that IL-6, a cytokine that can affect neuroendocrine mechanisms, plays a crucial role in the differentiation of B lymphocytes to antibody-forming plasma cells during the acute phase response and in hematopoiesis. There is a clear redundancy between the effect of this cytokine and those of leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, and IL-11. As shown by Dr. Hirano, this redundancy is related to the helical structure of proteins and to the fact that they share receptor subunits, particularly gp130, suggesting that there is a common use of intracellular transducing pathways.

I. Yamamoto (Okayama University, Okayama, Japan) reported that immune and neural functions are not only affected by classical immune-derived cytokines. He described how a stable ascorbate derivative (AA-26), which he developed as a source of vitamin C, is able to stimulate immune responses *in vitro*. This effect is potentiated by NGF which, when applied alone, can only marginally affect the immune response. Furthermore, AA-26 induces the expression of receptors for NGF in B lymphocytes. Conversely, although AA-26 has no effect on neurite outgrowth, it is able to potentiate the effect of NGF. These findings demonstrate how distinct molecules such as vitamin C and NGF can mediate interactions between cells of the immune and nervous systems.

E. Alleva (National Institutes of Health, Rome, Italy) presented an updated overview of the multiple biological roles exerted in adult mammals by NGF, a neurotrophin involved in homeostatic regulation of cross-talk among the nervous, endocrine, and immune systems. Animal models of stress have been used to establish that NGF is involved in mast cell activation. A human study using young parachutists found an increase in NGF serum levels the evening before their first sky-diving experience, in association with enhanced expression of NGF receptors in circulating mononuclear cells.

R. Glaser (Ohio State University, Columbus, OH) provided evidence that psychosocial stress can mediate the reactivation of herpes virus, as well as the EBV. This evidence was obtained from studies on the impact of academic stress in medical students during the examination period. Academic stress was associated with increases in antibody titers to EBV concomitant with down-regulation of various processes of the cellular immune response, such as the generation of specific anti-EBV cytotoxic and memory T cells. Classical stress hormones (*e.g.*, ACTH and corticosteroids) can mediate these effects because they can modulate the reactivation/replication of EBV *in vitro*. The combined effect of stressful conditions, viral reactivation, and immune suppression may increase the risk of EBV-induced tumors.

N. Hall (University of South Florida College of Medicine, Tampa, FL) discussed the possibility that early stimulation of neuroendocrine mechanisms as a result of perinatal infections results in long-lasting alterations in the response to stress. Exposure of 10-day-old rats to rat CMV led to a significant increase in both ACTH and corticosterone levels in a time- and dose-dependent manner. This viral activation of the HPA axis may well have been mediated by IL-1 in that this cytokine was also able to stimulate both ACTH and corticosterone release in these neonatal rats. It is significant that this activation occurred during the so-called hyporesponsive stress period, when it is relatively difficult to activate the HPA axis. When the CMV-exposed pups were assessed as adults, there was a decline in their corticosterone response to a novel environment. These studies indicate that the endocrine response to a stressor can be altered as a result of early viral exposure. Correlations have been reported between responsiveness to various stressors and the onset of certain types of tumors using a variety of experimental models. Thus, long-lasting alterations in the response to stress induced by neonatal exposure to CMV suggests the existence of a mechanism that can impact upon tumor progression in adult life.

S. Okamoto (Hokkaido University, Sapporo, Japan) reported that stimulation of the ventral medial hypothalamus led to a marked depression in lymphoproliferative responses to ConA, which was not affected by adrenalectomy, but which was prevented by severing the sympathetic innervation of the spleen. H. Furukawa (Hamamatsu University School of Medicine, Hamamatsu, Japan) referred to the lack of evidence for the idea that the HPA axis is important in the induction of neonatal tolerance.

K. Mašek (Institute of Pharmacology, Academy of Sciences, Prague, Czech Republic) reported the involvement of different brain areas in the immune response. On the basis of results of his group, he described the three circuits that seem to be involved in the immune response. One circuit involves the reticular formation and its catecholaminergic cell groups A_{1-7}, nucleus parabrachialis, and the central nucleus amygdala. These structures are mainly connected by means of descending neuronal pathways. A lesion in this circuit compromises the immune response. The second circuit involves the rapheal reticular formation with its serotonergic cell groups B_{6-8}, the hypothalamus, and the basomedial amygdala nucleus. These structures are connected mainly by ascending neuronal pathways. Lesions in this circuit enhance the immune response. The third circuit, which operates at the highest level, involves limbic telencephalic structures such as the medial frontal cortex and the septum, as well as the amygdala nucleus. Lesions in these mutually interconnected structures influence the immune response in both directions.

K. Hirokawa (Tokyo Medical and Dental University, Tokyo, Japan) presented an overview of aging in connection with the neuro-endocrine-immune network. He emphasized that the ability of this network to cope with stress is most efficient in young individuals in which all three of these systems are fully functioning. With advancing age, however, the functionality of these individual systems declines, and consequently, so does their ability to interact. The most important site for the concerted activity of the complex routes of this interaction is located in the hypothalamus. All signals from the outer environment enter the brain via the hypothalamus, which in turn, transmits signals to the endocrine and immune systems through various neurotransmitters or autonomic nerve fibers. Therefore, the hypothalamus appears to be one of the most important body clocks for determining the rate of development and aging of the neuro-endocrine-immune system.

Neuro-Immune Networks in Host-Tumor Relationships

In regard to the potential immunological mechanisms that may mediate the influences of the CNS on host defenses against cancer and other diseases, the predominant focus of attention at the symposium was on NK cell activity. There were thought to be two main reasons for the wide use of NK activity as an indicator of the immune response: (*a*) the relative ease of the assay and its ready applicability to virtually all experimental settings; and (*b*) evidence that NK cells play a major role in the body's resistance to cancer and viral diseases, particularly in regard to early responses and the ability of these effector cells to prevent metastatic spread of cancer. Some of the supporting evidence for this conclusion was presented by R. Herberman. He summarized evidence from studies using rodent tumor models that indicated that the rapid elimination of tumor cells from the circulation and the resistance to development of metastases are dependent on a functioning NK cell system. At the clinical level, he described several studies that indicated a significant correlation between levels of NK cell activity around the time of diagnosis of cancer and the metastasis-free interval after primary treatment of the disease. Dr. Herberman also presented recent evidence that experimental stress can have a biphasic effect on NK cell activity, with a very early rise and then a prolonged decrease. In several clinical studies involving both normal adults and cancer patients, difficulty in coping with stress was found to be associated with low NK activity and with relatively low resistance to disease, especially viral infections.

Several studies were presented at the symposium on how stress in experimental animals affects immunological function. Y. Okubo (Kyoto University, Kyoto, Japan) reported that foot shock and also stress induced depression in NK activity as well as gastric mucosal lesions. He further reported that administration of Chinese herbal medicines (Kampo) had some NK cell-dependent protective effects. M. Wenner (National Institute of Neuroscience, Tokyo, Japan) provided some indication of the location within the CNS where NK activity is modulated; 24 h after stimulation of the lateral hypothalamus, a center associated with reward/pleasure, there was an increase in NK activity, whereas stimulation of the ventral medial hypothalamus led to decreased NK activity. After ablation of the lateral hypothalamus, there was an initial increase in NK activity, followed by a prolonged decrease.

T. Hori (Kyushu University, Fukuoka, Japan) reported that injection of β endorphin into the brain reduced NK activity, and that this was blocked by naltrexone. IFN-α injection into the ventral medial hypothalamus led to increased firing, which was also blocked by naltrexone. IFN-α was shown to bind to brain opioid receptors; injection of IFN into the cerebral ventricles led to decreased splenic NK activity lasting for 0.5–2 h. Splenic denervation blocked the ability of interferon to decrease NK activity. Further indication of involvement of the sympathetic nervous system was obtained by stimulation of the splenic sympathetic nerves, leading to depressed NK activity, which was blocked by nadolol, a β-blocker. Immobilization stress also inhibited NK activity, which was largely blocked by splenic denervation.

A few studies using experimental animal tumor models were described that confirmed the role played by interactions between stress and the immune system on modulation of tumor growth metastasis. Y. Shavit (The Hebrew University, Jerusalem, Israel) reported decreased survival in rats bearing the MADB 106 tumor after exposure to an opioid stressor and reversal of this effect using naloxone. Morphine or other opioids also increased metastases from this tumor, and such treatment also inhibited NK activity. These opioid effects were shown to be dependent on actions of the CNS rather than directly mediated by the immune system. Some analogous effects of opioids on NK activity in patients were reported, with reversal of depressed NK activity by IL-2, and to a lesser extent, by IFN.

J. Kanno (Tokyo Medical and Dental University) described an interesting model of experimental stress induced in old mice by restraint or in young mice by parabiosis. Such stressors in aged mice led to more and larger metastases from the B16 melanoma. M. Suzuki (Basic Research Institute, Ajinomoto Co. Inc., Kawasaki, Japan) demonstrated an involvement of the neuroendocrino-immunological axis in the antitumor and anticachectic response to a host-mediated anticancer drug, lentinan.

Another approach for identifying neuro-endocrine-immune interactions during tumor growth was described by A. del Rey (Institute for Normal and Pathological Physiology, Philipps University). Studies by her group have shown that changes in the concentrations of insulin, thyroxin, prolactin, and corticosterone occur following inoculation of a few tumor cells of different types during *de novo* induction of a tumor in mice carrying the murine mammary tumor virus. These endocrine changes occur before the tumor becomes palpable. To explore which mechanisms mediate such effects, the EL-4 lymphoma was used as a model. The increase in corticosterone and decrease in insulin observed soon after inoculation of EL-4 tumor cells are caused by soluble mediators that require the presence of T lymphocytes in the host to exert their effects. Furthermore, histocompatibility between the tumor and the host is required for induction of the decrease in insulin levels. This evidence suggests the participation of the immune system in the mediation of neuroendocrine alterations during tumor growth. Furthermore, the increase in glucocorticoid levels following inoculation of EL-4 cells contributes to the inhibition of inflammatory mechanisms observed in animals bearing this tumor. As a whole, the data indicate that the presence of tumor cells is detected by the host and, as a consequence, a neuroendocrine response is elicited that can affect certain immune mechanisms and tumor progression.

T. Kato (Yamagata University) described studies indicating that short-term stressors, such as exercise or insufficient sleep, or the presence of dexamethasone, were associated with decreased sensitivity of neutrophils to apoptosis, whereas the stress induced by taking examinations was associated with increased neutrophil apoptosis.

Clinical Aspects of Psychoneuroimmunology in Cancer

PNI research in cancer is confronted with the challenge of a multilevel complexity imposed by psychological, neuroendocrine, and immune sequelae of the disease process itself, such as the secretion of cytokines by some tumors, or antitumor treatments that impair the QOL through their negative actions on the CNS. The research presented by N. Ohsawa (Osaka Medical College, Osaka, Japan) suggested that administering BRMs to cancer patients can reduce such undesirable side effects of cytotoxic cytokines as pain, fatigue and general malaise, and anorexia.

H. Kobayashi suggested that some of the modest but positive effects of BRMs on survival may be related to their antioxidant effects. However, R. Herberman injected a note of caution by pointing out that because most BRMs induce cytokines, which have a generally negative effect on QOL, it is important not to administer them in high doses but to establish the optimal levels for achieving the desired effects. As for the possible mechanisms involved in the improvement of QOL by therapy with BRMs, K. Ishikawa (Higashi-Sapporo Hospital, Sapporo, Japan) demonstrated the role played by the neuro-endocrine-immune network.

Most of the non-PNI psychological research conducted with cancer patients has addressed many of the QOL issues discussed in the presentation by T. Kawano (Toyoeiwa Women's College, Yokohama, Japan), including psychological and social adjustment, and disease or treatment-related symptoms such as pain, nausea, and loss of appetite. To the extent that factors with a negative impact on QOL constitute stressors, these factors can also influence the immune response.

Various types of acute and chronic stressors have been consistently associated with tumor development and growth in animal models. The situation for humans is, of course, vastly more complex, but it is a logical extension of the research connecting stressors with immune down-regulation and tumor progression in animals to hypothesize that psychological and behavioral coping responses, elicited in response to stress, can modify the impact of stress on immune parameters and subsequently, affect some points in the neoplastic process. To the extent that different patterns of coping are differentially adaptive with regard to modulating stress, certain patterns (*e.g.*, type C) may be associated with a less favorable cancer outcome.

The basis of the demonstrated association between NK cells and psychological phenomena has been the subject of much recent PNI investigation. D. Bovbjerg (Memorial Sloan-Kettering Cancer Center, New York, NY) presented a study on women with family histories of breast cancer, which has a high degree of inheritability. His results indicated that: (*a*) healthy women at familial risk have lower levels of NK cell activity than women at normal risk; (*b*) among women with family histories of breast cancer, there is an inverse correlation between the degree of familial risk and the level of NK cell activity; and (*c*) women at familial risk have higher levels of general distress and higher cancer-specific distress than do women at normal risk. The mechanisms underlying these associations, as well as the nature and direction of influence or causality, constitute an important subject for further research. These studies suggest the possibility of a genetic basis for interactions between certain emotional and immunological parameters.

Another type of immunological parameter, ATK activity, which is considered a positive prognostic indicator of solid neoplasms, was described by A. Uchida (Kyoto University) in terms of its relationship to physical and psychological stress. In two sets of experiments, ATK therapy of patients with primary localized solid neoplasms had the following effects: (*a*) induction of ATK activity at the time of surgery prevented tumor regrowth and metastasis; (*b*) adoptive transfer of BRM-induced ATK cells prolonged survival time, even in patients with metastatic or terminal disease; and (*c*) ATK activity was more strongly induced and maintained in patients who displayed less physical or psychological stress. It was hypothesized that when lower stress levels were modulated, by a positive outlook, ATK activity also promoted a longer survival time. The positive effects of increased ATK activity, irrespective of whether this was a result of "natural" ATK levels, BRM induction, or psychologically mediated induction, suggest that interventions for cancer might be made more beneficial by including psychological as well as biological components.

Type C, a coping style characterized by lack of emotional expression, particularly anger, and by the tendency to please others often at the expense of the self, was found in several studies by L. Temoshok (WHO, Geneva, Switzerland) to be related to melanoma progression. There is conceptual congruence between type C and the "stoic" behavior pattern identified by Greer and his colleagues, the "type 1"

personality described in the studies of Eysenck and Grossarth-Maticek, and the psychological trait of alexithymia, which signifies difficulty in experiencing and expressing emotions. N. Kawamura (National Institute of Neuroscience) found that scoring high on the Toronto Alexithymia Scale was associated with a low cytotoxic NK subset, as well as with a low cytotoxic T cell subset.

Consistent with these findings, the report by K. Zänker (University Witten/Herdecke, Witten, Germany) described how expression of anger was associated with a longer disease-free interval and survival in 50 breast and colon cancer patients. In addition, patients who scored low on curiosity showed earlier progression of disease. Lack of curiosity may indicate generally depleted or overwhelmed coping abilities, such that the person has little emotional energy left to invest in the outside world. It could be hypothesized that the positive effects of anger expression were mediated by the statistically significant decreases in ACTH secretion or reflected the positive correlation with β-endorphin levels. However, curiosity only showed a parallel negative correlation with ACTH, and was associated, unexpectedly, with fewer NK cells and $CD4^+$ cells in peripheral blood. K. Zänker elaborated on these conundrums in his presentation, in which he proposed that assessing various dynamic parameters of lymphocyte migration (speed, distance, time, directionality, and pattern) probably provides a more accurate picture of the functioning of lymphocytes, particularly $CD4^+$ cells, under the command of cytokines, than do assays of peripheral blood. Interestingly, this speculation is consistent with a previous report by L. Temoshok that malignant melanoma patients who expressed more emotion, including anger, at the time of biopsy had more lymphocytes at the base of the most deeply invaded portion of the primary tumor site, as determined by pathologist-rated assessments of paraffin-embedded sections from biopsies of primary lesions. Virtually all PNI oncology research has relied, however, upon assays of peripheral blood. K. Zänker's research on new methods to assess lymphocyte migration opens the door to new and potentially more valid evaluations of immunological function and intersystem communication.

At the end of the meeting, F. Sendo raised some issues related to the future development of this field of research. He first pointed out that basic studies on the neuro-endocrine-immune system have characterized this network in molecular terms. A number of studies have revealed associations between two or three of these systems. But we are far from understanding how the entire homeostatic system operates as a cohesive and coordinated unit. However, once a workable paradigm is established and new analytical methods become available for studying each segment of the network, this holistic understanding will enable us to map an entire response in terms of the sequential cascade of events involved in the various mechanisms. He also referred to the gap often perceived between psychology and other natural sciences. However, there is some evidence that this gap is narrowing. As an example, it has been shown that particular emotional changes induce the production of certain molecules and, conversely, that production of various molecules induces psychological changes. Differences in terminology across the various disciplines, which appear to be more linguistic than substantive, will hopefully decrease over time. F. Sendo concluded with the hope for increased dialogue among the various sciences involved in the field of PNI and expressed the shared wish of all the participants that this continuing cooperation will ultimately benefit patients suffering from cancer.

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