IMMUNOTHERAPY OF CANCER IN MAN

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Scientific Basis and Current Status

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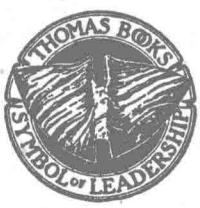

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FOREWORD

THE CONCEPT THAT CANCER is a disease resulting from or related to an immunologic derangement has intrigued investigators for more than 50 years. Motivating this continued interest was the possibility that once the immunologic responses to tumor growth and invasion were identified and understood, means could be devised to alter the balance against the tumor in favor of the host. Thereby cancer could be prevented or cured. Only in recent years has this "impossible" dream given promise of clinical application. The clinical discussion in this book shows how far we have come and what hopes we may have for the future.

Certain animal tumors regress when host immunity to the tumor is enhanced. However, specific antibodies (blocking antibodies) may protect the tumor from antitumor immune mechanisms of the host by interfering with the cytotoxic effect of immune lymphocytes on the tumor cells. There are also indications that this occurs in the development of human cancers. A good example of this is seen in neuroblastoma. Lymphocytes from children with neuroblastoma have the ability to kill neuroblastoma cells in culture. They also inhibit growth of neuroblastoma cells from other children with neuroblastoma. But serum from children with a progressively growing neuroblastoma almost invariably contains blocking antibodies able to nullify the inhibitory effect of the same child's immune lymphocytes on his tumor cells. Neuroblastomas with infiltrates of lymphocytes and plasma cells have a better prognosis than tumors which do not have infiltrates with these cells. Also, children with relatively high lymphocyte counts at the same time of diagnosis have had a longer survival and higher cure rate than those with low lymphocyte counts. Spontaneous regression of *in situ* neuroblastomas of infants and conversion of neuroblastomas into benign ganglioneuromas may be related to increased immune responsiveness of the host. These

findings suggest that an effective immune response to the tumor had taken place.

By bolstering immunologic responses in experimental animals, small foci of neoplastic cells may be eliminated. This indeed may be the eventual role of immunotherapy in cancer. After elimination of the primary mass of cancer cells by conventional means, we may then use immunotherapeutic techniques to rid the body of the few remaining malignant cells that otherwise would later give rise to recurrence of the cancer. If we could bolster the cellular hypersensitivity system sufficiently to eliminate the *last* cancer cell, or if we could remove blocking antibodies, we might be able to cure the disease.

Mathé has reported exciting clinical trials suggesting success in treatment of children with acute leukemia with immunotherapeutic methods. After inducing a complete remission using intensive combination chemotherapy, Mathé repeatedly injected BCG vaccine and a vaccine made from the patient's own leukemic cells, to stimulate the anti-leukemia immune mechanism. Very prolonged remissions were attained in many of the children so treated.

Cancer immunology and cancer immunotherapy are also tied closely with concepts regarding the viral etiology of cancer. Preventing or curing a cancer by providing a specific antiviral antibody seems to be another logical approach. Thus, Marek's disease, a virus-induced lymphosarcoma of chickens, is very effectively controlled with a live-virus vaccine. This is the first time that an animal cancer has been completely prevented by immunization.

Drs. Hersh, Gutterman, and Mavligit are very well qualified to write this book because of their many important original contributions in cancer immunology. They have comprehensively reviewed and succinctly analyzed a voluminous and complex literature. Their discussion is lucid and is oriented to increasing clinical application. This book will be of vital interest to all physicians who care for patients who have cancer.

CARL POCHEDLY

PREFACE

DURING THE LAST two decades we have seen a dramatic increase in our understanding of several important areas of mammalian biology, including the immunological system and the malignant process. Understanding of these two areas has converged in and permitted the development of the field of tumor immunology. Discoveries in the field have suggested that the presence of tumor antigens and tumor will permit the development of effective immunotherapy of human cancer.

While tumor antigens and tumor specific immune responses have been recognized in viral and carcinogen induced animal tumors for many years, there was until recently essentially no convincing evidence for the existence of these phenomena in human tumors. The few studies which had been done in man were generally ignored, in part because the evidence was circumstantial and in part because there was justifiable criticism of experimental design. It has only been in the last 20 years that the principles of cell-mediated immunity as applied to transplantation and cancer have been established. The role of cell-mediated immunity in the host control of tumors has now been well established. Tumor antigens and tumor specific immune responses have now been identified in the majority of human tumors which have been studied, and exciting preliminary experiments on the immunotherapy of human cancers have been conducted.

The clinical field of immunotherapy is embryonic. However, advances in basic and human tumor immunology and in conventional clinical cancer therapeutics strongly suggest that effective immunotherapy for human cancer will develop in the next few years. For this reason it seems that a review of the current status of immunotherapy in the context of the advances of the past two decades will serve several purposes. These will include orienting the cancer physician and the tumor immunologist to this overall field, introducing the biomedical community to the field and indicating the

medical and immunological guidelines along which rational and scientifically sound immunotherapy programs can be developed.

In this monograph we will attempt to analyze the current status of immunotherapy experiments in both animal systems and man. As a necessary background for this discussion, we will outline the current state of research on tumor antigens and tumor immunity in man and on the immunological deficiency associated with the pathogenesis and natural history of human cancer. No attempt to be encyclopedic has been made in this review. Rather, while much of the major literature is covered, we have been selective and have attempted to use examples of pertinent experiments and experimental approaches. At the end of the monograph, based on all the various studies reviewed, we indicate potential future pathways for the development of effective immunotherapy of human cancer.

The successful development of this monograph is largely due to efforts and insight of Dr. Carl Pochedly. The authors wish to thank him for his important help and advice. The authors also wish to thank Drs. Emil Frei, III and Emil J. Freireich for their continuing inspiration and support. We also thank Mrs. Judith Owens for her reference review and manuscript preparation.

EVAN M. HERSH

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Chapter One

IMMUNOLOGICAL DEFICIENCY IN CANCER

THE DISCOVERY OF TUMOR specific antigens and tumor specific immune responses in both animals and humans with malignant disease suggests that the status of the host defense mechanisms must be important in the etiology and pathogenesis of cancer. However, even before these tumor specific immunological phenomena were clearly documented in man, it was strongly suspected that host defense mechanisms or their failure played a role in etiology and pathogensis of tumors. As these tumor specific immune mechanisms were discovered in man, the concept of immunological surveillance was developed.[1]

Several well-known clinical phenomena have suggested the existence of this relationship. Spontaneous regressions of cancer have been observed both in patients with solid tumors and in patients with acute leukemia.[2] These have sometimes been associated with infectious complications and this has suggested an immunological mechanism. Pathologically, it has been observed that some solid tumors were associated with lymphocytic infiltration and more recently, the presence of lymphocytic infiltration in a primary tumor has been associated with a good prognosis.[3] Although there are many exceptions, the age relationships of cancer have also been used as evidence for host defense and immunological surveillance.[4] Thus, there are two peaks of high incidence of malignancy in man, during early childhood and during old age. These are periods of time during which, in both animals and man, immunological mechanisms are relatively weak. A final important clinicopathological ob-

servation is the very high incidence of *in situ* cancers and the relatively low incidence of related invasive cancers. Thus, histologic studies of the adrenals of young infants have shown a much higher incidence of malignant changes than the final incidence of clinical neuroblastoma in the population.[5] This suggests that immunological recognition of these *in situ* malignant transformations by the host defense mechanisms is responsible for the difference between the incidence of these lesions and frank malignancy.

Two sets of observations made more recently, strongly but indirectly support the concept that immunological deficiency is involved in the etiology and pathogensis of cancer. First, there is an increased incidence of malignancy associated with immunological deficiency diseases.[6] This includes such things as the increased incidence of lymphoma and leukemia among patients with various agammaglobulinemias and an increased incidence of a variety of malignancies in patients with ataxia telangiectasia. It also includes the increased incidence of malignancy in patients with various autoimmune diseases, themselves associated with immunological deficiency, such as Sjogren's syndrome and dermatomyositis. The second important observation is the recent disturbing finding that there is an increased incidence of malignant disease in patients undergoing chronic immnosuppressive therapy for the maintenance of organ allografts.[7] Thus, patients receiving daily oral prednisone and azathioprine have, in the first five post-transplant years, somewhere in the range of a 7 percent incidence of malignant disease, compared to 0.05 percent in the normal population. Many of these are reticulum cell sarcomas occurring in immunologically privileged sites, such as in the central nervous system, but most of the more common types of malignancy, such as carcinoma of the skin and cervix, have also been observed with higher than normal frequency.

Both of these pieces of evidence are indirect, and alternate explanations are available. Thus, it is conceivable that the same genetic basis for the immunological deficiency disease is the basis for an increased susceptibility to malignancy,

and the reduced host defense mechanisms have nothing to do with the pathogenesis. Similarly, it is possible that the chronic chemotherapy is mutagenic and an increased incidence of somatic mutation results in malignancy, rather than chemical immunosuppression. It is also possible that the tissue damage produced by the drugs activates oncogenic viruses already resident in the tissues.

In spite of these reservations, and in part on the basis of animal experiments to be described below, the concept of immunological surveillance has developed during the last few years. This concept was first given expression by Thomas, in a discussion on the general aspects of delayed hypersensitivity.[8] He states that "It is a universal requirement of multicellular organisms to preserve uniformity of cell type. . . . The phenomenon of homograft rejection will turn out to represent a primary mechanism for natural defense against neoplasia." Since that initial statement, Sir McFarlane Burnet has greatly expanded and popularized this concept in a series of lectures and papers. His concept can be stated as follows: "In large, long-lived animals, like most of the warm-blooded vertebrates, inheritable genetic changes must be common in somatic cells and a proportion of these changes will represent a step towards malignancy. It is an evolutionary necessity that there should be some mechanism for eliminating or inactivating such potentially dangerous mutant cells and it is postulated that this mechanism is of immunological character."[1]

Direct evidence to support the surveillance hypothesis has come from a variety of animal experiments. Of prime importance are the studies of the immunological consequences of chemical or viral carcinogenesis in mice. Susceptible mice, subjected to either viral[9] or chemical[10] carcinogenesis, undergo a period of moderate to profound immunological suppression, during the latent period before the development of identifiable tumors. This immunological imcompetence involves both cell-mediated and humoral immunity and, interestingly, tends to recover as the primary tumors first appear. This is a general and not a tumor specific

immune defect. In animals resistant to chemical and/or viral carcinogenesis, such immunological suppression is not observed.[11]

Even more important are the effects of specific types of immunosuppressive treatment on the incidence and rate of development of primary or secondary neoplasms of known etiology in animals. Thus, neonatal thymectomy,[12] antilymphocyte serum,[13] or immunosuppressive drug treatment can increase the rate of development and incidence of viral and chemical carcinogen-induced and spontaneous tumors. In addition, these treatments will accelerate the rates of local growth and metastasis of already established tumors.[14] In these cases, there can be no question of genetic effects, since the etiology of the tumors is known. One must conclude that the specific effects of these treatments, particularly on cell-mediated immunity, are responsible for tumor dissemination. This is the strongest support for the concept of the surveillance mechanism. Final proof for the validity for this hypothesis that tumor immunity in both animal systems and in humans, as far as it has been studied, seems identical to classical transplantation immunity. No differences between transplantation immunity and tumor immunity have been found. Therefore, it is almost certain that the original hypothesis of Thomas, as expanded by Burnet, is correct.

Immunological deficiency is involved not only with the etiology but also with the pathogenesis and entire natural history of the malignant process. Now that we are in the era of systemic treatment, recent studies indicate that immunological mechanisms are also involved in the response to conventional nonimmunological treatment. This will be expanded in the paragraphs below, but at this time it is necessary to state that if this is true, no consideration regarding immunotherapy or *any* form of therapy can be made without taking into account the immunological status on the subject. The immunological deficiency associated with cancer is of two types. First, there is nonspecific immunological deficiency which becomes more severe as the disease disseminates. Second, there is tumor specific immunological

deficiency, related to the release of tumor antigens which operates to suppress local host defenses, even when only the primary tumor exists. This may be one of a number of reasons why primary cancers disseminate even when the host is apparently immunocompetent. Early dissemination may be because of this local mechanism while late dissemination is also due to the nonspecific mechanism.

Immunological deficiency associated with cancer (see Table I), that is, associated with established malignant dis-

TABLE I
HOST DEFENSE FAILURE IN CANCER PATIENTS

	Disease Category							
	Solid Tumor				AL[3]		Hodgkin's	Multiple
Immunological Parameter	Good Prog.	Poor Prog.	CLL[1]	CML[2]	Good Prog.	Poor Prog.	Disease	Myeloma
1° Antibody response	N[4]	D[5]	D	N	N	N (D)[6]	D	D
2° Antibody response	N	N	D	N	N	N	N	D
Ig levels	N	N	D	N	N	N	N	I[8], D
Blastogenic response								
PHA[9]	N	D	D	?[10]	N	D	D	D
Antigen[11]	N	D	D	?	N	D	D	D
MLC[12]	N	D	D	?	N	D	D	D
1° D.H.[13] response	N	D	N (D)	N	N	D	D	N (D)
2° D.H. response	N	D	N	N	N	D	D	N
Mediator[14] production	N	?	D	?	?	?	D	?
Inflammatory response[15]	N	D	N	D	D	D	D	?

[1] CLL—Chronic lymphocytic leukemia
[2] CML—Chronic myelogenous leukemia
[3] AL—Acute leukemia, all types
[4] N—Normal
[5] D—Diminished
[6] ( ) —Slightly
[7] Ig—Immunoglobulin
[8] I—Increased
[9] PHA—Phytohemagglutinin
[10] ?—Not evaluated sufficiently
[11] Antigen—To which subject immune
[12] MLA—Mixed lymphocyte cultures
[13] D.H.—Delayed hypersensitivity
[14] Mediators—Such as migration inhibitory factor and lymphocyte cytotoxin
[15] As measured by the skin-window technique

ease, was observed first in patients with lymphoid malignancies and only more recently in patients with solid tumors. Immunological deficiency has been studied most extensively in patients with Hodgkin's disease. An early observation

was that these patients had increased susceptibility to certain types of infections, specifically fungal disease and tuberculosis. A suspicion that they had impaired cell-mediated immunity arose from the fact that they had this increased incidence of tuberculus infections.[15] Subsequently, it was shown by Aisenberg that patients with Hodgkin's disease had an impaired ability to develop new delayed hypersensitivity to a sensitizing antigen such as DNCB (dinitrochloro-benzene).[16] The penomenon was most prominent in patients with active and disseminated disease and was not present in patients without active disease. In parallel studies, Aisenberg found that these patients had a normal antibody response to pneumococcal polysaccharide.[17] This led him to hypothesize that these patients had a specific defect in cell-mediated immunity. The observation that these patients had impaired ability to reject homografts[18] and they had impaired lymphocyte transfer reactions[19] as well as low lymphocyte counts[20] tended to support this hypothesis. However Chase, in a careful review of a large number of immunological studies in these patients,[21] pointed out that they had normal ability to mount secondary antibody responses but a markedly impaired ability to mount primary antibody responses. This was later confirmed by Hersh and co-workers.[22]

More recent studies aimed directly at lymphocyte function, tend to confirm the complexity of the immunological defect. *In vitro,* these patients' lymphocytes have diminished ability to respond to mitogenic stimulation and to undergo lymphoblastoid transformation.[2] This is due both to an intrinsic defect and to a serum factor. In addition, after mitogenic stimulation, they have an impaired ability to release cytotoxin.[24] Conversely, the circulating lymphocytes without stimulation, release more cytotoxin than do normal human lymphocytes.[24] A number of these studies have confirmed the fact that these immunological defects in Hodgkin's disease correlate directly with the extent of disease and disappear as the patients enter remission. However, the underlying defect remains completely unknown. It is possible that identification of sub-populations of lymphocytes of

bursal and thymic origin will permit us to further investigate the nature of the underlying defect.

Immunological deficiency is also characteristic of patients with chronic lymphocytic leukemia (CCL), and has been studied extensively.[25] These patients have an impaired ability to mount a primary and secondary response, and as their disease progresses and their lymphocyte count rises, their immunoglobulin level falls. In spite of this, they have normal established delayed hypersensitivity and it is uncertain whether they have impaired or normal primary delayed hypersensitivity.[26] Conflicting data exists on this point. At some stages of their disease, they may actually have augmented delayed hypersensitivity reactions, particularly to mosquito bites.[27] They do have a lymphocytic defect, which can be demonstrated *in vitro*. Their lymphocytes responded poorly to mitogenic stimulation.[28] This correlates inversely with the height of the white blood-cell count. The higher the count, the poorer the response. If the patients are brought into remission, and the count returns to normal, the lymphocyte response returns to normal. This poor response is now known to be due to dilution of the normal number of lymphocytes by the abnormal leukemic lymphocytes. They have a normal number of recirculating thoracic duct lymphocytes.[22] Recent studies, using fluorescent antibody techniques to detect immunoglobulin determinants on the lymphocyte surface (B cells) indicated that most patients have 100 percent of their circulating lymphocytes positive compared to 8 to 36 percent in normal subjects.[29,30] Studies with fluorescent labelled antibodies against specific immunoglobulin allotypes indicates that these abnormal lymphocyte populations are clonal in origin. This suggests that chronic lymphocytic leukemia (CLL) is an abnormal proliferation of a B cell clone. This would explain the specific defects in antibody production and would suggest that CCL is a disease not dissimilar to multiple myeloma in its etiology.

Immunological deficiency in the other lymphoid malignancies has been less well characterized. In multiple myeloma, there is a progressive diminution in the primary and