Tumor Immunology

Charles F. McKhann, M.D. and Melvin A. Yarlot, Jr., M.D.

Tumor Antigens

All cells have on their surface normal transplantation antigens, which reflect individual specificity and induce rejection of transplanted tissues. However, when a normal cell becomes malignant, it often undergoes biochemical changes that result in the production of new cellular antigens, sufficiently aberrant as to be recognized by the host, causing an immune response. (Fig. 1.) These new antigens, which may be present in any part of the cell, most significantly are often found on the surface where they may interact with other host cells, particularly those responsible for immune recognition. Tumor antigens on the cell surface are dispersed among normal transplantation antigens, probably in a reciprocal relationship, so that a cell that has many tumor-specific antigens will be deficient in transplantation antigens and vice versa.¹ In experimental systems, these new antigens are found on all tumors induced by chemical carcinogens, oncogenic viruses or foreign bodies. To a lesser extent, they are found on some “spontaneous” tumors, but there is no evidence to suggest that they are absolute components of cancer, present on all malignant cells. Current knowledge of the biochemical nature of these antigens is very limited. Not only part of the “bricks and mortar” of the cell membranes, they may include enzyme systems and other materials of major functional importance to the cell.

The new antigens may result from outside information brought to the cell in the form of a virus, or from mutations in the native information of the cell DNA, as induced by a chemical carcinogen. These tumor-specific antigens are highly specific for the individual tumor or, in the case of many virally induced tumors, common to all tumors caused by the same virus.

In addition, tumor-associated antigens may be present in several different types of cancers, as well as some normal tissues. The now familiar carcinoembryonic antigen (CEA) fits this category. These antigens may be malignant expressions of latent information that the cell has suppressed from embryonic stages. Many tumors appear to have both tumor-associated antigens, which they share with other tumors and even normal tissues, as well as antigens that are highly specific for the individual tumor. Regardless of their origins, the new cellular antigens elicit a response in the host that clearly indicates recognition of the abnormal cell.

¹ Dr. McKhann is Professor of Surgery and Microbiology, Department of Surgery, University of Minnesota, Minneapolis, Minnesota.
Dr. Yarlot is Clinical Fellow, Department of Surgery, University of Minnesota, Minneapolis.
The Immune Response

The immune response to an antigenic tumor, like that to a kidney allograft, has two major components. The first, a cellular immune response, produces circulating lymphocytes capable of destroying tumor cells on contact. These lymphocytes originate as stem cells in the bone marrow but mature in the thymus, from which they derive the popular name, T-cells. T-cells reside in all lymphoid organs and undergo prompt division and release into the circulation upon stimulation by antigen. T-cell activity against experimental and human tumor cells has been measured by various in vitro functions, including lymphocyte stimulation and cellular cytotoxicity. Lymphocyte stimulation represents the "recognition" phase of the immune response and is measured by the proliferation of T-cells cultured with whole tumor cells or various preparations of tumor antigens. T-cell stimulation and proliferation may occur by direct interaction of tumor antigen with an immunocompetent T-cell. However, T-cell activation occurs primarily through intimate contact between a T-cell and a macrophage which has previously absorbed and possibly processed the antigen.

Another T-cell function, cellular cytotoxicity, represents the "effector" phase of the immune response and is measured by the extent to which stimulated lymphocytes can damage or destroy cultured tumor cells. These lymphocytes may operate by releasing cytotoxins which act over a short distance, or by direct contact with the tumor cells, to create holes in the membrane, eventually resulting in lysis and death of the malignant cell.

Another cell of importance, which collaborates with the T-cell in tumor destruction, is the macrophage. Through an elaborate set of humoral signals, the macrophage is first attracted to the immune lymphocyte (chemoactic factor), then immobilized in its vicinity (Macrophage Inhibiting Factor–MIF), and finally instructed or activated by the lymphocyte (Macrophage Activating Factor–MAF). The activated macrophage is a relatively nonspecific killing cell but one which appears to have the remarkable ability to selectively kill malignant cells with which it comes in contact.

The second major component of the immune response, antibody production, results from activation of a different population of lymphoid cells, the
B-cells. These cells also originate from stem cells in the bone marrow but, unlike T-cells, mature in the bone marrow and, possibly, in the wall of the intestinal tract or spleen of mammals. When stimulated by antigen, B-cells proliferate and differentiate into plasma cells, which are the major source of antibody production. B-cells may be stimulated directly by tumor antigen or indirectly by "helper" T-cells. Antibody directed against a specific tumor can be detected by its absorption onto the tumor cells utilizing isotope- or fluorescein-labeled antibody, complement fixation, or by cytotoxic death of the tumor cells when complement is present as well as antibody.\(^4\) There is also evidence of an interaction between lymphoid cells and antibody that is lethal for tumor cells. In this case, the specificity of the interaction is governed by the antibody which is absorbed into the target cell. A subclass of lymphocytes known as K-cells, probably a variant or immature form of B-cells, is bound by the antibody, and can then destroy the target cell.

The several cell types involved in the immune response interact in an almost "orchestral" fashion, exchanging elaborate signals at both cellular and humoral levels. Primary stimulation of the immune response by a tumor appears to take place through T-cells, B-cells or both, depending on the nature of the antigen. Macrophages may also function at this level by processing the antigen and increasing its potency for subsequent use by lymphocytes. Both suppressor and activator functions have been attributed to T-cells, such as the capacity to recruit other T-cells and to cooperate with B-cells in promoting antibody production. A diagram of the interrelationships of some cellular components of the immunologic system can be found in Figure 2. (See page 194.)

Rejection of both tumors and transplanted tissues appears to be mediated primarily by T-cells. While some tumors, particularly those of lymphoid origin are very sensitive to the cytotoxic effects of antibody and complement, most solid tumors, such as sarcomas, are relatively unaffected by these circulating mediators and under certain circumstances may even receive some protection from exposure to antibody or antibody-antigen complexes.

**Human Tumors**

A wide variety of human tumors have been shown to be antigenic (Table 1), for example, Burkitt's lymphoma of Central Africa, sarcoma, melanoma, neuroblastoma of infancy, the lymphoreticular tumors, and carcinomas of the colon, breast and bladder. These tumors appear to share common tumor antigens within their histologic groups, but not between different groups. They all seem to induce both cellular and humoral immunity. Burkitt's lymphoma, the first human tumor shown to be antigenic, has served as a prototype for subsequent studies of other tumors.\(^5\) Sera from patients with Burkitt's tumor contain antibody directed against tumor cells, detected by immunofluorescence. Such positive sera frequently react with Burkitt cells from other patients with the disease, but rarely with cells from patients with another type of tumor or from normal controls.

Antigen extracts of cell membranes from a long-term Burkitt's lymphoma cell line (RAJI) have been used to immunize rabbits. The antiserum produced was cytotoxic not only for RAJI cells but also for peripheral lymphocytes of patients with acute lymphocytic leukemia and acute myelogenous leukemia, but not to lymphocytes of normal individuals or leukemia patients in complete remission. Immunization of patients in remission with RAJI cell membrane fractions has induced antibody cytotoxic for allogeneic leukemia.
cells, as well as for their own cells collected before induction of remission and stored in a frozen state. These findings indicate that Burkitt’s lymphoma is recognized as antigenic by the patient and that tumor cells from one patient share some antigens with cells from other patients with Burkitt’s disease as well from those with acute leukemia. This relationship is commonly seen in experimental tumors induced by viruses. Indeed, a putative virus has been isolated from Burkitt’s lymphoma cells on many occasions, although definite proof that it is the causative agent is still lacking. It is noteworthy that the virus recovered from Burkitt’s tumor cells appears to be similar, if not identical, to the virus responsible for the common benign disease, infectious mononucleosis.

A solid tumor of entodermal origin that has also received a great deal of attention is carcinoma of the colon and rectum. Gold and associates were the first to isolate and characterize an antigen from this tumor. They inoculated rabbits with extracts of colon carcinoma and obtained antibody directed against a tumor antigen even after exhaustive absorption with normal colon tissue. The antigen detected was found on cells from colon carcinoma and normal fetal gut in the first two trimesters of gestation, thus the name, carcinoembryonic antigen (CEA). This antigen is a protein-polysaccharide complex and is found on the cell surface where it is released into the circulation. Radioimmunoassays capable of measuring nanogram quantities of CEA now make it possible to detect abnormally high levels of this antigen in the sera of patients with a variety of gastrointes-
tinal and other tumors, as well as some benign gastrointestinal diseases. Despite its lack of specificity for colon carcinoma, CEA determinations prove valuable in following tumor patients for evidence of preclinical recurrence. Serum levels of CEA usually fall to near normal levels following removal of a tumor, but become elevated again in the presence of recurrent disease.

Cell-mediated immunity to human tumors is demonstrated in vitro by the cell inhibition assay, which measures growth inhibition of tumor cells when cocultivated with lymphocytes from the same patient or from a patient with a histologically similar tumor. In reality, the tumor cells are probably killed by

Table 1. Antigenic Human Tumors

| Tumors                        |
|-------------------------------|
| Burkitt’s Lymphoma            |
| Neuroblastoma of Infancy      |
| Malignant Melanoma            |
| Osteogenic Sarcoma            |
| Liposarcoma                   |
| Leukemia                      |
| Retinoblastoma                |
| Wilms’ Tumor                  |
| Renal Cell Carcinoma          |
| Carcinoma of Skin             |
| (Basal and Squamous Cell)     |
| Carcinomas of                 |
| Colon                         |
| Lung                          |
| Stomach                       |
| Esophagus                     |
| Breast                        |
| Thyroid                       |
| Parotid Gland                 |
the immune lymphoid cells. If the number of tumor cells surviving after cultivation is significantly lower in cultures with the patient’s lymphocytes, compared to cultures with normal lymphocytes, the patient is said to have cell-mediated immunity against his tumor. In a large series,7 cellular immunity was detected in 88 percent of 373 patients with a variety of solid tumors when the peripheral blood lymphocytes were tested against the patient’s tumors; it was present in an equally large number of patients when the lymphocytes were tested against similar tumors from other patients. Tumor antibody has also been detected in the circulation of many cancer patients. These studies indicate that many human cancers contain tumor-associated antigens that elicit detectable immune responses.

Immunity and Cancer

It has been proposed that the first line of defense in preventing the development of tumors may be “immunologic surveillance,” which recognizes as foreign, small numbers of malignant or premalignant cells and destroys them before a true cancer can develop.8 Any compromise of the immune system would interfere with its surveillance capacity. Indirect evidence for this theory emerged from studies with immunologically deficient patients9 and those undergoing renal allografts whose normal immune responses have been suppressed deliberately.10 In both groups, the incidence of cancer is far above normal. (Table 2.) Although the concept of immunologic surveillance is appealing, at best it is probably only a secondary factor in the capacity to resist tumor development. The spectrum of cancers seen in patients with abnormal immune responses is somewhat weighted toward mesenchymal tumors, and does not reflect those seen in normal populations of the same age. Moreover, following inoculation of animals, very small numbers of tumor cells that should be vulnerable to immunologic surveillance often “sneak through” and become established. In such systems, “spontaneous” tumors are of relatively low antigenicity and the immune system does not respond promptly.

Investigators of tumor immunology have been plagued with the question of how tumors thrive, even in the presence of well-documented host immunity. Although occasional pulmonary metastases seem to melt away following removal of the primary tumor, particularly in renal cell carcinoma, spontaneous regression of clinical tumors remains a rarity.

It is well recognized that many conventional forms of cancer therapy, particularly chemotherapy, irradiation and also, to a lesser extent, surgery are associated with generalized suppression of the immune response. This is manifested by anergy to common skin test antigens, resistance to DNCB sensitization and poor lymphocyte responsiveness in vitro to mitogens such as PHA.

It is now also clear that many growing tumors exert a suppressive effect on the general immune capacity of the host, even prior to therapy.11 This was first noted in Hodgkin’s disease and is now recognized in many other tumors. Although immunosuppression is more pronounced in patients with advanced cancer, it can also be seen in relatively early tumors even when cachexia is not a problem and the total tumor burden is modest.

At the present time it is not known whether generalized immunosuppression is found more frequently in some tumors than in others. Several observers have noted that continuing impairment of the host’s general immune capacity is associated with a poor prognosis. Nonspecific stimulation of the immune system with such substances as BCG has been able to reverse
immune suppression and produce a more favorable outlook for the patient. Indeed, this may be an important mechanism and the basic justification for nonspecific immunotherapy.

**Circulating Blocking Factors**

Suppression of the immune response by the tumor also has its highly specific components. The killing capacity of cytotoxic lymphocytes can be demonstrated by placing the attacking and the target cells together in culture. However, this is an artificially restrictive situation compared to the intact organism, as the addition of serum from the tumor-bearing individual frequently results in the abrogation or “blocking” of lymphocyte-mediated cytotoxicity. Two mechanisms, which have been demonstrated in experimental systems, may explain this phenomenon. Antibody directed against some cancers, particularly solid tumors, appears incapable of damaging the malignant cells and may actually protect them from cytotoxic lymphocytes. In practice antibody-antigen complexes, rather than free antibody, are probably present in the circulation; according to recent evidence, these complexes may block cellular immunity at the surface of the target cells. Conversely, free antigen produced and released by the tumor cell into the circulation has also been found a potent weapon for disarming the immune response. Such antigen, produced in quantity “for export,” can saturate the specific immune response in the lymph nodes, bone marrow, spleen, thymus or circulation to the extent that the immune system is no longer interested in the tumor. The extent to which these mechanisms overlap or dominate each other in the natural interaction of tumor and host is now being extensively investigated.
Immunotherapy

The ultimate goal of tumor immunology is successful treatment of the cancer patient. Clinical trials currently in progress have been initiated largely as a result of findings that: (1) nonspecific augmentation of the general immune system can cause tumor destruction; (2) tumors sharing common antigens can be used interchangeably in unrelated individuals to promote tumor immunity; (3) alterations of the surface of tumor cells may render them more immunogenic; and (4) the immune response, even under optimal experimental conditions, is not capable of dealing with large amounts of tumor. This latter point has two corollaries: immunotherapy should be intensive, and the tumor burden of the host should first be reduced to a minimum by conventional therapy.

As has been shown clearly in experimental systems, immunotherapy carries the undesirable potential for actually promoting or "enhancing" tumor growth. The major factors involved are apparently related to over-stimulation of antibody production with subsequent circulation of large amounts of antigen-antibody complexes or possibly free antibody. Based on the knowledge that the tumor, as well as conventional forms of therapy, may reduce the general immune capacity of the host, it has often been stated that immunotherapy should not be attempted in immunologically impaired individuals. However, at present, nonspecific immunotherapy's mechanism of action is still poorly understood, and it is quite possible that reversal of this inhibition may be one of its important functions.

Current approaches to immunotherapy fall into three major categories: nonspecific, specific and transfer of immunity. (Fig. 3.) Nonspecific immunotherapy utilizes materials that have no antigenic relationship to the tumor, but appear to increase the general immune capacity of the individual. Several micro-organisms have been found capable of stimulating the general immune capacity. Of these, BCG (Bacille Calmette-Guérin)\textsuperscript{14} and Corynebacterium parvum are receiving the most extensive clinical trials. In experimental systems, these agents are capable of preventing tumor growth and aborting the growth of small established tumors. Nonspecific immunotherapy seems to promote both cell-mediated immunity and antibody production and may be effective in partially reversing the immunosuppressive effects of both the tumor and conventional therapy. The most extensive trials with BCG are directed toward acute leukemia, malignant melanoma and soft tissue sarcomas. The results with acute myelogenous leukemia suggest that BCG may be of clinical value, while those with acute lymphatic leukemia remain contradictory. This may be due, in part, to the significant improvement in induction and maintenance regimens of chemotherapy which have occurred in the course of some of these trials. Local recurrences of melanoma have been treated by the intrallesional injection of BCG, with a reported regression rate of 90 percent of the injected nodules.\textsuperscript{15} Unfortunately, regression of un.injected adjacent nodules, which would be strong evidence for effective systemic immunization, was only 17 percent. BCG has also been frequently used in conjunction with immunotherapy directed specifically at the tumor.

Specific immunotherapy turns an immunologic "spotlight" on the tumor, without necessarily augmenting the host's general immune capacity. It employs substances that are antigenically related to, or products of, the tumor such as: killed tumor cells from the same patient or from another patient with an antigenically similar tumor; tumor cells that have been altered in vitro, rendering them more immunogenic; and antigen preparations ex-
enables immunocompetent B-cells to respond to antigens which they otherwise are unable to recognize. Macrophages may also transport antigenic material that has undergone processing by the macrophages themselves and also by T-lymphocytes to the immunocompetent B-lymphocytes. The stimulated B-lymphocytes differentiate into plasma cells which are the main producers of antibody.

A population of cells, possibly variants of the B-cell termed Killer cells (K), are able to attack tumor target cells only after the tumor cells have been exposed to specific antibody. Proliferation and release of blastogenic factor are not required for the development of individual cytotoxic T-lymphocytes, but are important steps in increasing the number of cells capable of dealing with the tumor.

Not shown in the diagram are cells responsible for immunologic "memory," and an important set of feedback controls, again mostly centered around sensitized T-lymphocytes, through which inhibitory subpopulations of these cells suppress the production of sensitized lymphocytes and antibody-forming cells.
tracted from tumor cells. Attempts to modify and increase the immunogenicity of tumor cells are currently under way using the enzyme neuraminidase, which removes a protective coating of sialic acid from the cell surface. The use of soluble antigen extracts for immunotherapy is being approached with caution until the role of free antigen as a potential blocking factor is better understood. The nature of this material, the form in which it is presented to the patient and the route of administration may all be very important.

The transfer of tumor immunity was first attempted by transferring lymphocytes from one tumor patient to another, frequently between “paired” patients following cross-immunizations with each other’s tumor cells. These potentially immune lymphocytes have one drawback: they are foreign and easily rejected by the recipient. However, they undeniably have the capacity to prevent tumor growth in experimental systems that do not reject them. Even when rejected, immune lymphoid cells may transfer information to the recipient’s cells that will increase reactivity against the tumor.

More recently, steps have been taken to transfer tumor immunity at the informational level itself, free of foreign lymphoid cells. Two sources of infor-
Fig. 3. The three major approaches to immunotherapy are active nonspecific immunization, active specific immunization, and transfer of immunity. In each case nonimmune lymphoid cells (I) are converted to specifically or nonspecifically sensitized cells (II) which are then capable of attacking the tumor. Specific immunotherapy requires inactivating or killing the tumor cells and then returning the killed cells to the host in multiple injections. The cells may be altered with such materials as neuraminidase, which removes a surface coating of sialic acid thus increasing immunogenicity, or cell-free antigen extracts made from the tumor cells. Nonspecific and specific immunization are carried out in the original tumor-bearing individual, while transfer of immunity delivers the specific immune capacity to another individual with a similar tumor by intact lymphoid cells or with extracts of immune RNA or transfer factor made from such cells.
mational molecules recovered from immune lymphoid cells are undergoing investigation: immune RNA and transfer factor. Their actions are quite similar, although they are not identical materials and their ultimate relationship is unknown. In particular, it is not known whether the information conveyed is indeed specific for the tumor, or whether they are potent but nonspecific promoters of some component of the immune mechanism. Both immune RNA and transfer factor can be used to stimulate lymphoid cells in vitro, or they may be injected directly into the recipient. Unlike intact lymphoid cells, they are nonimmunogenic and therefore induce no host immune response that brings about their own destruction. Transfer factor appears to effect cellular immunity exclusively and not antibody production, a selectivity that may be of great significance in tumor immunotherapy. Finding a source of transfer factor or of immune RNA is, however, a cause of concern. The "cured" patient is an ideal donor of lymphoid cells from which these materials can be prepared. However, with some tumors such as melanoma and carcinoma of the breast, identification of the "cured" patient is difficult even at five or more years. In addition, it is theoretically possible that active immune resistance against a few residual tumor cells may be important to these patients. Removal of large numbers of lymphoid cells for production of immune RNA or transfer factor could conceivably upset an important immunologic balance.

**Summary**

At the present time the interaction between the immune response of the tumor-bearing individual and his cancer is undergoing intense investigation. This has already provided a great deal of information about the etiology and biology of many tumors. The major goals, however, are earlier diagnosis and effective immunization as a new mode of therapy. The current dramatic expansion in our understanding of the immune system offers every hope that these goals will eventually be achieved with many tumors.

**References**

1. Haywood, G. R., and McKhann, C. F.: Antigenic specificities on murine sarcoma cells: Reciprocal relationship between normal transplantation antigens (H-2) and tumor-specific immunogenicity. J. Exp. Med. 133: 1171-1187, 1971.
2. Martz, E., and Benacerraf, B.: An effector-cell independent step in target cell lysis by sensitized mouse lymphocytes. J. Immun. 111: 1538-1545, 1973.
3. Greaves, M. F.; Owen, J. T., and Raff, M. C.: T and B Lymphocytes. New York: American Elsevier Publishing Co., Inc., 1973.
4. Mann, D. L.; Leventhal, B., and Halterman, R. L.: Human antisera detecting leukemia-associated antigens on autochthonous tumor cells. J. Nat. Cancer Inst. 54: 345-347, 1975.
5. Klein, G.; Clifford, P.; Klein, E., and Stjernswärd, J.: Search for tumor-specific immune reactions in Burkitt lymphoma patients by the membrane immunofluorescence reaction. Proc. Nat. Acad. Sci. U.S.A. 55: 1628-1635, 1966.
6. Gold, P., and Freedman, S.O.: Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J. Exp. Med. 121: 439-462, 1965.
7. Hellström, I.; Hellström, K. E.; Sjögren, H. O., and Warner, G. A.: Demonstration of cell-mediated immunity to human neoplasms of various histological types. Int. J. Cancer 7: 1-16, 1971.
8. Burnet, F. M.: Immunological aspects of malignant disease. Lancet 1: 1171-1174, 1967.
9. Kersey, J. H.; Spector, B. D., and Good, R. A.: Immunodeficiency and cancer. Advances Cancer Res. 18: 211-230, 1973.
10. Penn, I., and Starzl, T. E.: A summary of the status of de novo cancer in transplant recipients. Transplantation Proc. 4: 719-732, 1972.
11. Burk, M. W.; Yu, S.; Ristow, S. S., and McKhann, C. F.: Refractoriness of lymph node cells from tumor-bearing animals. Int. J. Cancer 15: 99-108, 1975.
12. Hellström, K. E., and Hellström, I.: Lymphocyte-mediated cytotoxicity and blocking serum activity to tumor antigens. Adv. Immun. 18: 209-277, 1974.
13. Baldwin, R. W. et al.: Inhibition of hepatoma-immune lymph-node cell cytotoxicity by tumor-bearing serum, and solubilized hepatoma antigen. Int. J. Cancer 11: 527-535, 1973.
14. Mathé, G. et al.: Active immunotherapy for acute lymphoblastic leukemia. Lancet 1: 697-699, 1969.
15. Morton, D. L., et al.: BCG immunotherapy of malignant melanoma: Summary of a seven-year experience. Ann. Surg. 180: 635-643, 1974.
16. Simmons, R. L., et al.: Immunospecific regression of methylcholanthrene fibrosarcoma with the use of neumaminidase. Surgery 70: 38-46, 1971.