Immunological Approaches to CANCER Therapeutics

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A Wiley-Interscience Publication
JOHN WILEY & SONS
New York • Chichester • Brisbane • Toronto • Singapore

Preface

Considerable progress has been made since 1950 in the management of various types of human neoplasias with chemotherapeutic agents alone or in combination, or with other modalities of treatment. Patients with certain tumors can be brought into complete remission by chemotherapy or radiotherapy and are free of detectable disease five years or longer after diagnosis. In other cases, adjunctive treatments after surgical excision of primary tumors significantly increase the percentage of long term survivors. The list of tumors which in some cases can be successfully treated include acute leukemias, lymphomas—particularly Hodgkin's disease—certain types of choriocarcinoma, testicular tumor, Wilm's tumor, certain tumors of the skin, osteosarcoma of the limb, and certain clinical types of breast tumors, ovarian carcinoma, oat cell carcinoma of the lung, and thyroid tumors.

Despite the obvious advances achieved, major limitations must still be overcome before effective treatment of many neoplastic diseases can be achieved. Chemotherapy, for example, suffers from such limitations as insufficient selectivity for the target tumors and toxicity to normal tissues. Therefore, in most cases a relatively minor degree of tumor resistance to a drug cannot be overcome without unacceptable toxicity. Many approaches are being pursued in efforts to develop more effective anticancer treatments; these include the development of new drugs and new modalities of treatments based on increased knowledge of the biochemical and biological basis of selectivity of antitumor action as well as the development of new types of treatment that may modify the interactions between host and tumor.

Based on the assumption that tumor-associated antigens would elicit relatively specific host responses, many attempts have been made recently to develop immunotherapies to utilize this potential. It was postulated that these treatments would have minimal toxicity, because they were to exploit physiological host reactions, and could be used without additional toxicity in cooperation with less selective cytoreductive modalities. Largely because of the empiricism which pervaded the design of the early immunotherapy trials, the initial optimism, which was reinforced by what later appeared to have been premature reports, was followed by substantial skepticism about the potential value of this approach. However, solid evidence has indeed been obtained in experimental systems and, to a limited extent, in humans indicating that alteration of tumor-host relationships may be therapeutically exploitable. It is therefore appropriate to review the knowledge gained in this area so that the potentialities and limitations of this approach can be carefully assessed. The

purpose of this book is to provide a critical review of the experience gained to date in the pursuit of immunological approaches in cancer therapeutics and to propose areas in which opportunities for further development can be reasonably expected to be realized in the future.

With the acquisition of more knowledge concerning the mechanisms of immune functions and their regulation, it now seems possible to develop new approaches in immunotherapy and to better utilize the new agents that recently have become available. It has become increasingly apparent that many of the immunomodulating, immunostimulating, and immunorestorative agents may cause different effects depending on the status of immune systems at the time they are exposed to these agents. It is reasonable to postulate, therefore, that the success of a certain immunotherapeutic regimen may depend partly on the development and appropriate utilization of methodologies to assess the status of antitumor host defense systems in individual patients. Through this assessment it should be possible to acquire further knowledge of the regulation of the immune response in humans. This would provide additional opportunities for the development of improved treatments.

The existence of tumor-specific or tumor-associated antigens is a central prerequisite for achieving antitumor selectivity through the exploitation of specific immune defense mechanisms against tumor. Knowledge in this area is expanding. However, the existence of tumor antigens does not necessarily imply that an effective antitumor response can be elicited; indeed untoward immune responses and/or tumor escape mechanisms need to be understood if effective immunotherapy is to be designed. Active immunotherapy with tumor cells or a tumor antigen preparation has been a major goal of immunotherapy which to date has escaped full realization in the face of the difficulties surrounding the identification of tumor-specific antigens. Immunotherapy with modified tumor cells, which stimulate a host response, has been achieved in numerous animal systems and is being verified in humans.

Modification of immune responses to tumor may be obtained through augmentation or restoration of the effector mechanisms of host defenses, or through immunomodulation leading to the establishment of a favorable balance between therapeutically desirable and undesirable components of host reactions. Such modifications may be achieved through the use of microbial products, chemical compounds, thymic hormones and interferons, or nutritional control. The possibility of transferring immunological responsiveness through the administration of such products as immune RNA or Transfer Factor might deserve future study toward possible applications in cancer therapeutics.

The use of hybridomas as "factories" of monoclonal antibodies has a potential in therapeutics which is just beginning to be studied. In fact, based on the assumption that tumor has distinctive antigens, monoclonal antibodies directed against them may provide, upon passive transfer, the means of attacking the tumor directly, or may serve as a vector for selective delivery of cytotoxic agents to the tumor. The transfer of cells represents an area where major progress has been made both in terms of reconstitution of immune po-

tential through the therapeutic transfer of bone marrow cells to heavily irradiated and immunosuppressed patients and in terms of adoptive transfer of immune lymphocytes. The latter approach has been facilitated recently by the isolation of specific factors allowing the growth in culture of sensitized cells which can then be used in adoptive transfer trials.

Although the clinical value of most of the immunotherapeutic regimens tested to date has not yet been unequivocally proven or has been found to be at best relatively limited, it is reasonable to expect that treatments capable of increasing the efficacy of host responses to tumor will be developed and will ultimately provide essential tools in the definitive management of certain forms of cancer. To this end, it is essential to acquire further basic knowledge of the host defense systems and the mechanisms by which they may be perturbed, particularly in humans. It also seems important to continue to develop new agents aimed at the exploitation of immune systems in cancer therapeutics. I hope that the information discussed in this book will be a source of stimulation and a basis for rational planning toward the further development of novel cancer immunotherapies.

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Buffalo, New York
March 1982

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Serologic Analysis of Human Solid Tumor Antigens

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1. INTRODUCTION

A central issue in studies of human tumor immunology deals with whether human cancer cells express antigens not present in normal adult tissues of the host. The identification and characterization of these putative tumor antigens may be of use in the development of new diagnostic and therapeutic strategies and could significantly add to our understanding of malignant transformation and the host response to it.

The existence of tumor-associated antigens has been clearly established in many experimental animal systems. Animal experimentation has utilized primarily *in vivo* manipulations such as immunization and tumor cell challenge. The use of inbred animal strains and transplantable tumors induced by carcinogenic agents and viruses has provided valuable reproducibility of both host and tumor. A variety of antigen types have been described in experimental tumors (Table 1). With the use of *in vivo* transplantation techniques, tumors induced with chemical carcinogens have generally been found to express unique antigens, although antigens common to a variety of tumors have also been detected (1). Serologic (antibody) techniques have also been utilized to detect tumor antigens in carcinogen-induced tumors, and both unique and cross-reacting antigens have been described (2). In contrast to carcinogen-induced tumors, tumors induced by the same virus have been shown most often to express common or virus-associated group-specific antigens (3,4). Antigens normally expressed only during fetal development have been detected in many animal tumors, including those induced with chemical carcinogens as well as with viruses (5,6).

Human tumors have also been extensively analyzed over the last decade for the presence of tumor-associated antigens. In studies in humans, of course, it has been impossible to employ the kinds of *in vivo* transplantation test with viable cells that have formed the basis for the identification of tumor-associated antigens in animal systems. Although some studies have involved *in vivo* skin

TABLE 1. Types of Antigens that May Be Present on Experimental Tumors

Tumor associated
Unique
Common
Viral (etiologic)
Fetal
Tissue specific
Allospecific
Species specific
Artifactual (method of antigen preparation, culture conditions, contaminating virus, etc.)

testing with nonviable tumor cells or antigen preparations, most have relied on *in vitro* tests of lymphocyte or antibody reactivity to tumor cells or antigen preparations. A major problem with human testing has been the difficulty in obtaining sufficient and reproducible quantities of tissue. This has led to the extensive use of cultured tumor cells as a source of antigen. As is discussed more thoroughly in a subsequent section, the use of cultured cells has been accompanied by a host of problems that have impeded progress in this field.

Despite these difficulties, many serologic studies have claimed the demonstration of tumor-associated antigens in human tumors. An important aspect of the serologic study of tumor antigens is the simultaneous testing of both tumor tissues and normal adult tissues to define the tumor specificity of the involved antigens. Much of the early work in this field did not sufficiently address questions of tissue specificity. In many instances when putative tumor-associated antigens were more thoroughly studied and more sensitive assays developed, the antigens were detected, sometimes in lower quantities, in normal adult tissues as well. Many other serologic analyses, however, have provided seemingly strong evidence for the existence of tumor-associated antigens in human malignancy. In this chapter we present an overview of serologic studies of human solid tumors, with particular emphasis on melanoma and sarcoma.

2. METHODS OF ANALYSIS

2.1. Sources of Antigens and Antibodies

Whole tissues, tissue-cultured cells, tissue homogenates, and various soluble antigen preparations have been used as sources of human tumor antigens. Although cultured cells provide a convenient and reproducible source of antigen, several problems are associated with their use (Table 2). Irie et al. (7,8)

TABLE 2. Problems with Use of Tissue-Cultured Tumor Cells in Serologic Studies of Human Tumor Antigens

Identification of cells as malignant (overgrowth of normal cells)
Change of cells with passage (overgrowth of tumor subpopulations)
Expression of neoantigens not expressed *in vivo*
Loss of *in vivo* antigens on establishment in tissue culture
Altered antigenicity with variations in
Culture conditions (medium, serum source, CO_2 concentration, temperature)
Growth stage (cell cycle, state of confluency, time from last medium change)
Use of proteolytic enzymes in cell preparation
Binding of medium components to cells (heterologous serum antigens)
Contamination of cultures with antigenic organisms (PPLO, viruses, etc.)

described the ability of cultured cells to incorporate foreign antigens present in heterologous sera used in culture media, and such antigens are a potential source of confusion in serologic analyses. The expression of antigens can be highly variable, depending on cell cycle and culture conditions (9–13). An even greater problem is the difficulty in establishing the malignant nature of the cultured cells. Although various approaches have been taken, including growth of cells in immunosuppressed animals, karyotype analysis, ultrastructural studies, marker analysis, and growth characteristics in culture, no single criterion appears sufficient to characterize a cultured cell as malignant (13). Normal cells may overgrow in cultures derived from tumor tissue, and this finding may be a source of confusion in many studies.

Antibodies to human tumors have been obtained both from human sources and after immunization of animals with various tumor preparations. Although sera from cancer patients have been a major source of human antibodies, elution of antibodies from tumor specimens has also been achieved (14). Antibodies from human as opposed to xenogeneic sources are of particular interest because they may reflect a response by the host to immunologically relevant tumor antigens. However, there are drawbacks to the study of human tumor antigens with the use of sera from cancer patients. Human sera have generally been found to yield low concentrations of antibodies, and highly sensitive tests are required for their detection. Such tests have often been confused by the finding that normal human sera possess antibodies directed against certain antigens present on the cell surface (15). Xenogeneic antisera, on the other hand, can usually be obtained with much higher titers. However, such preparations react with a variety of nonrelevant antigens, and extensive absorption with normal tissues must be performed. A recent approach to overcome this problem has involved the use of somatic cell hybrids to produce high-titered monoclonal antibodies reacting with a single antigenic specificity (16). Although such work is still in early stages, this technique has great potential for obtaining large quantities of highly specific antibodies against tumor-associated antigens.

2.2. Methods of Assay

Various methods have been used to detect antibody binding to tumor antigens (Table 3), and a compendium of this methodology has recently been compiled (17). The most extensively used assay has utilized immunofluorescence to measure antibody binding to the surface of viable cells and to the cytoplasm and nucleus of fixed cells. Several other highly sensitive assays that also measure antibody binding to cell membranes involve the use of indicator red blood cells, as in the immune adherence and mixed hemadsorption tests. Assays involving the ability of bound antibody to induce cell lysis include complement-dependent cytotoxicity and antibody-dependent, lymphocyte-mediated cytotoxicity. Complement fixation and immunoprecipitation assays have been used in many studies to detect reactivity to solubilized antigens. Recently,

TABLE 3. Assays Used in Serologic Analysis of Human Solid Tumor Antigens

Assays measuring antibody binding to cells
Immunofluorescence (direct and indirect)
Immune adherence
Mixed hemadsorption
Isotopic antiglobulin
Protein A (radiolabeled and erythrocyte tagged)
Assays measuring antibody function
Complement-dependent cytotoxicity
Antibody-dependent cell-mediated cytotoxicity
Assays utilizing soluble antigen
Complement fixation
Immunoprecipitation (immunodiffusion, immunoelectrophoresis, radioimmunoassay)

radioimmunoassays have been employed in many different systems to measure antibody binding to both cells and solubilized antigens. These assays can be highly sensitive and are also readily quantifiable, an advantage over more subjective tests such as those involving immunofluorescence or indicator cells that must be assessed by microscopic examination.

Depending on the assay and the reagents employed, a test will be sensitive only for certain classes of immunoglobulin. For example, an indirect immunofluorescence assay may be designed to detect IgG antibodies, whereas a complement fixation assay will be sensitive to those immunoglobulin classes able to fix complement, primarily IgM and IgG. Because important reactivities might be missed with single assays, the use of a battery of serologic tests has been recommended (18).

Absorption analyses, utilizing both whole tissues and solubilized antigens, have been a common feature in most serologic studies. Such analyses allow for the detection of tissue antigens that cross-react with those present in the test preparation and thus are vital to determining antigen specificity. Absorptions are also useful with tissues that are not suitable for direct tests. In addition, absorption analyses may detect antigens that were not detected in direct tests (19). Absorption analyses have drawbacks, however. Tissue preparations may nonspecifically absorb immunoglobulins or release substances that interfere with the assay. Furthermore, the preparation of tissues for absorption may alter their antigenicity. These problems continue to plague most serologic studies.

3. ONCOFETAL ANTIGENS

Some tumors appear to express antigens or synthesize proteins normally expressed only by fetal and not by adult tissue. Many of these oncofetal antigens

have been described, including serum proteins, cell surface antigens, intracellular enzymes, and ectopic hormones [recently reviewed by Sell (5) and Uriel (6)]. However, most of these fetal antigens are not strictly tumor-associated, and with the use of sensitive assays, they have also been detected in small amounts in nonmalignant adult cells. The two best-studied oncofetal antigens are alphafetoprotein (AFP) and carcinoembryonic antigen (CEA). These antigens have been analyzed with the use of xenogeneic antisera raised against tumor or fetal preparations. Alphafetoprotein is a serum protein associated with normal fetal and neonatal development and with the growth of hepatocellular tumors (5,6). However, it is also produced during liver regeneration, and low-serum AFP levels have been found in the normal adult. Carcinoembryonic antigen is a fetal colon cell-surface glycoprotein that is produced by tumors of ectodermal origin—intestinal, pulmonary, pancreatic, gastric, and mammary adenocarcinoma (5,6). Elevated serum CEA levels are also associated with smoking and with inflammatory diseases of bowel, lung, and pancreas. Low-serum CEA is present in normal adults.

Several oncofetal antigens have been described that appear to be specific for fetal and tumor tissue, although more extensive analyses or more sensitive tests may identify such antigens on normal adult tissues. Avis and Lewis (20), using an approach similar to that used to detect CEA, raised rabbit antisera to perchloric acid extracts of human fetuses. The sera, which were absorbed with normal tissue components, reacted by membrane and cytoplasmic immunofluorescence with fetal tissues and with certain human tumors. No reactivity was observed with several normal adult tissues.

This concept of a common oncofetal antigen was further supported by the work of Irie et al. (21). Using an immune adherence assay, those investigators found that sera from several melanoma patients reacted with a melanoma cell line in tissue culture. An extensive absorption analysis indicated that a common antigen was present on the surface of a variety of histologic types of biopsied and cultured tumors and on human fetal brain. A number of biopsied normal tissues were negative for the antigen. However, when muscle and skin were placed in tissue culture, they became positive for the antigen, indicating that normal adult tissues may reexpress fetal antigens under conditions of tissue culture. This common oncofetal antigen (OFA) appeared to be immunogenic because melanoma patients receiving therapy with allogeneic melanoma cells bearing OFA displayed increased titers of anti-OFA (22). However, anti-OFA reactivity has also been found in normal individuals (23).

A similar fetal-associated antigen has been described by Rosenberg and coworkers with the use of a complement-dependent cytotoxicity assay. Most human sera were found to possess antibodies reactive with common antigens expressed on cultured sarcomas, cultured normal skin fibroblasts, and first-trimester human fetal tissue (24–26). Normal adult tissues prior to culture did not express fetal antigens. Similar antibody levels were found in sarcoma patients when compared to normal individuals (24,27), and this finding raises several possibilities: (1) the fetal antigen may cross-react with a common en-

vironmental antigen; (2) the antifetal reactivity may be the result of an immune surveillance mechanism against malignant transformation in normal individuals; or (3) the reactivity may be an autoantibody directed against an undetected normal tissue antigen.

Salinas et al. (28,29) have presented evidence for the existence of a common interspecies OFA. With the use of radiolabeled antiglobulin and membrane immunofluorescence assays, human sera were found to react with both human and mouse fetal liver cells. Sera from patients with various types of cancer showed a markedly higher incidence of reactivity (71–92%) than did normal sera (11–31%). However, because an extensive absorption analysis was not performed, the tissue specificity of the xenogeneic antigen was not well established.

Blood group antigens are determined by carbohydrate chains present on the cell surface. The loss or gain of these chains is a common occurrence in fetal erythrocytes. Several studies have demonstrated, in isolated cases, the appearance of illegitimate blood group antigens, that is, normal blood group antigens that are nevertheless different from those present on normal tissues of the host (30). T antigen, the precursor of blood group MN antigens, is an antigen not found on normal adult erythrocytes. Springer et al. (31) demonstrated the presence of T antigen on all breast cancers studied, whereas benign mammary glands taken from the same individuals did not express T antigen. Anti-T antibodies were found in all human sera tested.

Numerous other studies have identified OFAs. Many of these are discussed in the following sections dealing with specific tumor types.

4. MELANOMA ANTIGENS

The antigenicity of malignant melanoma has been more extensively studied than that of any other human solid tumor. This may be due in part to the clinical impression that immunologic factors play a role in the natural history of the disease. Little evidence exists to support this impression, however. The immunology of melanoma has recently been reviewed (32,33).

4.1. Tests with Human Sera

Membrane Antigens. Many workers have utilized human sera as a source of antibody to study melanoma cells for the presence of cell-surface tumor-associated antigens (Table 4). Several classes of antigen have been detected: unique antigens reactive only with autologous sera, common antigens cross-reactive with some or all other melanomas tested, and OFAs. Lewis and coworkers (34,35), using cytotoxicity and membrane immunofluorescence tests, detected reactivity in patient sera directed solely against autologous melanoma cells. The antibodies could be absorbed only with autologous tumor, and not with autologous normal cells or allogeneic tumor. Immunofluorescence and

TABLE 4. Detection of Cell Surface Melanoma Antigens with Use of Melanoma Patient Sera

Assay	Antigen-Positive Tissues	Antigen-Negative Tissues	Comments	Ref.
Immunofluorescence	Fresh autologous and allogeneic melanoma		Fifty-seven percent of melanoma patients and 13% of normal sera reactive	40
Cytotoxicity, immunofluorescence	Fresh and cultured autologous melanoma	Allogeneic melanoma, autologous skin	One-third of patients sera gave autologous reactions	34, 35
Cytotoxicity, immunofluorescence	Cultured autologous melanoma	Most cultured allogeneic melanoma, autologous skin, epidermal and colonic tumors	Normal sera unreactive; several melanoma sera reacted weakly with allogeneic melanoma	36
Cytotoxicity	Cultured allogeneic melanoma and breast carcinoma		Thirty percent of melanoma patients and 7% of control sera reactive to melanoma	42
Immune adherence	Cultured autologous and allogeneic melanoma	Allogeneic skin fibroblasts, lymphocytes, cultured breast carcinoma line	Eighteen percent of melanoma patients and no normal or other tumor sera reactive with allogeneic melanoma	10
Complement fixation	Fresh autologous and allogeneic melanomas	Fresh sarcoma, carcinoma, normal muscle, kidney, lung	Test antibodies were eluted from fresh melanomas	14
Cytotoxicity	Cultured autologous melanoma	Most cultured allogeneic melanoma	Fifty percent of patient sera gave autologous reactions; 14% of positive sera reacted with allogeneic melanoma	37
Immune adherence	Fresh and cultured melanoma and other tumors, cultured normal skin, muscle	Fresh normal skin, muscle, other tissues, fresh and cultured lymphocytes	Termed *oncofetal antigen* (OFA)	21

TABLE 4. (Continued)

Assay	Antigen-Positive Tissues	Antigen-Negative Tissues	Comments	Ref.
Mixed hemadsorption, immune adherence, anti-C3 mixed hemadsorption, protein A	Three classes of antigen detected I Unique antigen on autologous cultured melanoma only II Common antigen on autologous and allogeneic cultured melanoma only III Normal antigen on various fresh and cultured normal and malignant cells of human and animal origin		Studies utilized melanoma patient sera reactive with autologous tumor	18, 19, 49, 50
Cytotoxicity	Cultured allogeneic melanoma		Thirty-four percent of melanoma patients and 21% of normal sera reactive	41
Immunofluorescence, mixed hemadsorption	Autologous and allogeneic cultured melanoma	Autologous and allogeneic fibroblasts, cultured nonmelanoma tumors, human and monkey kidney, fresh fetal	Melanoma patient reactivity seen only after immunization with autologous or allogeneic melanoma cells	52, 53
Immune adherence	Cultured allogeneic melanoma, brain tumor, adult fibroblasts, fresh human fetal	Red blood cells, pooled platelets	Melanoma sera were preabsorbed with red cells and platelets	55
Radioimmune precipitation	Two membrane antigens solubilized from a fresh melanoma 80,000 MW[a] allogeneic 124,000 MW autologous		Four normal sera unreactive with solubilized antigens	38, 39

TABLE 4. (Continued)

Assay	Antigen-Positive Tissues	Antigen-Negative Tissues	Comments	Ref.
Immunofluorescence, immune adherence	Autologous and allogeneic cultured melanoma	Autologous and allogeneic fibroblasts and lymphoblasts	Melanoma sera were preabsorbed with virus-transformed fibroblasts or lymphoblasts	54
Immunofluorescence	Two antigens detected	Fresh autologous and allogeneic skin and kidney		45
	OFA—autologous and allogeneic fresh and cultured melanoma, other cultured tumors and fibroblasts, fetal brain			
	After fetal brain absorption—autologous and allogeneic fresh and cultured melanoma	Sarcoma, breast carcinoma, fibroblasts, fetal brain		
Immune adherence, complement fixation	Two antigens detected		Test antibodies isolated by serum-affinity chromatography on melanoma membranes	46
	OFA—autologous and allogeneic fresh and cultured melanoma sarcoma, carcinoma, fibroblasts	Fresh liver, lung, lymphoblastoid cell line		
	After absorption with sarcoma—autologous and allogeneic melanoma	Sarcoma, fibroblasts		
Complement fixation, immune adherence, protein A radioassay	Two antigens isolated from melanoma culture medium		Fifty-six percent of melanoma patient sera and 12 to 23% of normal and other tumor sera reacted with TAA	47, 48
	OFA-cultured allogeneic melanoma and fibroblasts, fetal brain	Allogeneic lymphoblastoid cell line		