Tumor Immunology and Its Application to the Diagnosis of Cancer

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ABSTRACT

There are several types of immunological approaches to the diagnosis of cancer. The search for circulating tumor associated antigens has been used most extensively. Carcinoembryonic antigen (CEA) and alpha-fetoprotein are the best studied examples. In addition, several hormones and other proteins have shown some association with the neoplastic state. Other immunodiagnostic approaches include the study of the general immune competence of cancer patients and the cell-mediated and humoral immune response to tumor associated antigens. Although several assays seem promising, none have been adequately evaluated as to their sensitivity and specificity. The potential role of these tests in screening for cancer, in differential diagnosis and in monitoring of the cancer patient after therapy remains to be determined.

Introduction

Host resistance to the growth of neoplastic cells has been a subject of intense interest for many years. The recent demonstration that many tumors of animals and also of man have tumor associated antigens has provided a firm experimental basis for this resistance. The major directions in tumor immunology have been the study of the individual’s immunological response to these antigens and the mechanisms for increasing protection against tumor growth. In addition, however, the concepts and findings in tumor immunology have very important applications to the immunodiagnosis of cancer.

The types of potential immunodiagnostic approaches are summarized in table I. The first approach, the detection of tumor associated antigens, has been utilized most extensively. Tumor cells may contain antigens which are undetectable or are present in smaller amounts in normal cells. On this basis, antibodies in patients, sera, or in the sera of animals immunized against these antigens, can potentially be used to discriminate between tumor cells and normal cells. To be useful in immunodiagnosis, the tumor antigens must be common to a variety of tumors, at least of the same histological type. Studies in animal model systems have shown that tumors induced by chemical carcinogens and some spontaneous tumors have tumor associated antigens which are individually distinct and are not present in other tumors induced by the
same agent. Such antigens would not be helpful diagnostically, since antibodies against one tumor could not be expected to react with any other tumors. Common tumor antigens have been identified and, in general, can be classified into three categories: (1) virus induced: tumors induced by the same virus, even when they differ in morphologic appearance, share some of the same tumor associated antigens; (2) fetal or carcinoembryonic antigens: antigens present on normal fetal cells may also be expressed on a variety of tumor cells, regardless of etiology; and (3) tissue antigens: normal tissue or organ associated antigens may be expressed in large amounts in tumor cells derived from that tissue.

Tumor Antigens

Many human tumors have been found to contain common tumor antigens. Antibodies against these antigens could be used to examine tissue biopsies or exfoliated cells. At present, this potentially useful approach has not been extensively explored. Most of the emphasis has been on the detection of antigens released from the tumor cells and present in the circulation or in secretions. The carcinoembryonic antigen (CEA) of Gold is the best studied example of this.

Depression in the immune competence of the cancer patient might also be useful diagnostically. Decreased immune surveillance has been suggested as an important factor in cancer development. Tumor growth can also produce immunosuppression. In either case, decreased immune competence compared to normal or benign disease populations has diagnostic implications.

Many tumor associated antigens can elicit an immune response in the tumor-bearing individual. Antigens can often be recognized when present in very small amounts, and it might, therefore, be expected that immunological reactions could be detected while tumors were still small and localized. Both cell-mediated immune responses and humoral antibodies can be measured. In addition, specific serum factors, which block cellular immune reactions, can be identified.

These various immunological approaches could be applied to several different diagnostic problems. The most general application would be for screening of population groups, particularly those at high risk of developing cancer. A more restricted role, but in some ways more immediately practical, is the use of immunological tests in the differential diagnosis of patients with symptoms or signs suggestive of cancer. For this purpose, the immunological techniques would serve as adjuncts to the conventional laboratory diagnostic procedures. Another major area of potential application is the monitoring of patients with cancer, both during and after therapy. Immunological techniques could provide prognostic information or give early evidence for residual or recurrent disease. For practical use of immunodiagnostic procedures, the two main issues are their sensitivity and specificity. In the context of this discussion, sensitivity can be defined as the frequency of positive tests with cancer patients. Specificity is the frequency of positive tests with controls, either those with benign disease or normal individuals. As with nonimmunological tests, assays with high sensitivity, particularly in early stages of disease, and high specificity will be most useful. Other important criteria for diagnostic applicability
are the reproducibility and ease of performance of a test, and particularly the feasibility for large scale testing.

**Current Status of Immunodiagnostic Tests for Cancer**

The various circulating antigens which have been suggested for possible diagnostic application are shown in table II. CEA, as measured by radioimmunoassay, initially appeared highly promising. More recent studies have indicated some problems in its sensitivity and specificity. The basic underlying problem may be that CEA is not strictly a fetal antigen or a tumor associated antigen, being present in nonmalignant adult tissues and in normal plasma. Its use in discriminating between tumor-bearing and non-tumor bearing individuals may depend on the relative concentrations of CEA in neoplastic tissue versus other tissues, and the degree of disruption of the barrier to leak of CEA into the bloodstream. Tests for CEA can differentiate fairly well between sera from known tumor patients and those from normals and benign disease controls. The main issue to be resolved for CEA is its degree of reliability in making this distinction in patients with early, previously undiagnosed disease and in cancer patients with the question of small amounts of residual or recurrent disease. It is the opinion of the author that more studies are needed to answer this.

Alpha fetoprotein (AFP) is in a similar stage of development as CEA. It was initially thought to be specific for tumor-bearing individuals; however, with the more sensitive radioimmunoassay, AFP also appears to be present in small amounts in normal serum. Patients with hepatoma may have very high elevations in AFP levels. In the United States, more important applications may be for the differential diagnosis and monitoring of patients with choriocarcinoma, testicular cancer and possibly gastric cancer. As with CEA, the practical usefulness of the tests for AFP for reliable early diagnosis and for monitoring tumor growth has not yet been sufficiently documented.

Research on the other circulating antigens shown in table II is not as far along, although an increasing amount of information is accumulating for most of these. It is quite possible that no one assay for circulating antigens will be a sufficient diagnostic tool. However, with increasing numbers of such assays available, one can envision a panel of several tests being used, providing additive or even synergistic information for diagnosis.

**Decreased Immune Competence**

The types of assays for decreased immunological competence which might be useful in the study of cancer patients are summarized in table III. Skin tests for delayed hypersensitivity have been used most extensively. Many studies have involved tests with recall antigens, to which many or most individuals have been naturally sensitized. Impaired reactivity has been seen mainly in cancer patients with advanced disease. Tests for delayed hypersensitivity, which involve sensitization to an antigen to which the individual had not been previously exposed, and then sub-

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### Table II

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<thead>
<tr>
<th>Circulating Antigens Potentially Useful in Immunodiagnosis of Cancer</th>
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<tr>
<td><strong>1. Fetal antigens</strong></td>
</tr>
<tr>
<td>A. Carcinoembryonic antigen (CEA)</td>
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<tr>
<td>B. Alpha fetoprotein (AFP)</td>
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<tr>
<td>C. Gamma fetoprotein</td>
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<tr>
<td><strong>2. Hormones</strong></td>
</tr>
<tr>
<td>A. Human chorionic gonadotrophin (HCG)</td>
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<tr>
<td>B. Human placental lactogen (HPL)</td>
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<tr>
<td>C. Parathyroid hormone</td>
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<tr>
<td>D. &quot;Big&quot; ACTH</td>
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<tr>
<td><strong>3. Other</strong></td>
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<tr>
<td>A. Tal T-globulin</td>
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<tr>
<td>B. Regan isoenzyme (alkaline phosphatase)</td>
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sequent challenge, appear to be more sensitive indicators of immune depression. Failure of cancer patients to be sensitized to dinitrochlorobenzene (DNCB) has been found to be frequently associated with unresectable disease or early recurrence after surgery.14,45,60

*In vitro* assays for cell-mediated immune reactivity have also been used to look for decreased reactivity in cancer patients. Decreased lymphocyte stimulation by mitogens has been extensively studied, but this defect has been seen mainly with advanced disease.24 The lymphocytes of many cancer patients, some with early, localized disease, have been found to have decreased cytotoxic reactivity.53,46 Another *in vitro* assay which holds promise to be a sensitive index of cellular immune depression in cancer patients is that of rosette formation with sheep erythrocytes (E rosettes). Thymus-derived lymphocytes (T cells) have been found to form E rosettes, and the assay appears to reflect the proportion of T cells in peripheral blood. The majority of cancer patients studied before therapy, with either localized or metastatic disease, had decreased percentages of E rosette forming cells.61,62

None of these assays for immune competence has been adequately tested for its potential use in the immunodiagnosis of cancer. Prospective studies of symptomatic patients or populations with increased risk of cancer are needed to evaluate their role in diagnosis.

**Immune Response to Tumor Associated Antigens**

In addition to general immune competence of cancer patients, many studies of the immune response to tumor associated antigens have been performed as shown in table IV. Despite the frequent depression in cell-mediated immunity, many patients with tumors have been shown to have detectable immune responses to these specific antigens. Skin tests for delayed hypersensitivity reactions to extracts of tumors have been performed in a manner similar to that employed with common bacterial antigens.8,23 Reactions to antigens common to many tumors of the same histological type have been observed in acute leukemia,8 intestinal cancer,25 breast cancer,2 lung cancer26,60 and cervical cancer.60 Reactivity to leukemia associated antigens has been shown to correlate with clinical state,8 and skin tests with tumor extracts may be generally useful for prognosis and for monitoring response to therapy. The skin tests also have potential application to screening for cancer. The major difficulty lies in the possible hazards associated with the inoculation of extracts from cancer tissues into individuals without cancer. Since the skin reactive antigen in intestinal cancer has also

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**TABLE III**

**ASSAYS FOR DECREASED IMMUNE COMPETENCE AS POSSIBLE DIAGNOSTIC PROCEDURES**

<table>
<thead>
<tr>
<th>1. Skin tests for delayed hypersensitivity</th>
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<tr>
<td>A. Recall antigens</td>
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<tr>
<td>B. Primary sensitization: DNCB, KLH</td>
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<tr>
<td>2. Enumeration of circulating T and B Lymphocytes</td>
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<td>A. Rosette assays</td>
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<tr>
<td>B. Immunofluorescent and cytotoxic antibodies</td>
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<td>3. Lymphocyte functions</td>
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<tr>
<td>A. Transformation</td>
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<tr>
<td>B. Cytotoxicity</td>
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<td>4. Macrophage functions</td>
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**TABLE IV**

**ASSAYS FOR IMMUNE RESPONSE TO TUMOR ASSOCIATED ANTIGENS**

<table>
<thead>
<tr>
<th>1. Cell-mediated immunity</th>
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<tr>
<td>A. Skin tests for delayed hypersensitivity, using extracts of tumor cells</td>
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<tr>
<td>B. Cytotoxicity assays, against tumor-derived target cells</td>
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<tr>
<td>C. Leukocyte migration inhibition, using extracts of tumor cells</td>
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<tr>
<td>2. Humoral factors</td>
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<tr>
<td>A. Antibodies against tumor antigens (e.g. immunofluorescence, cytotoxicity)</td>
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<tr>
<td>B. Factors which block assays of cell-mediated immunity</td>
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</table>
been found in normal fetal intestine and liver, the fetal extracts should provide safe materials for such testing. Fetal lung extracts have also given positive reactions in some patients with carcinoma of the lung. Fetal antigens from various tissues may be generally useful for diagnostic testing.

Cell-mediated cytotoxicity assays also have potential applications to immunodiagnosis. The Hellströms and others have reported that the lymphocytes of patients with various types of cancer can inhibit colony formation or can cause lysis of tissue culture cells derived from the tumors. In general, the reactivity has been against antigens common to tumors of a particular histological type. If only individuals developing tumors had cell-mediated reactivity against these antigens, a sensitive detection method might be available. The central issue is the amount of normal reactivity in the assay. Hellström et al reported that lymphocytes from patients with diseases other than neuroblastoma or from normal individuals did not react against cell lines derived from neuroblastomas. However, they and others have noted some reactivity of normal individuals against tumor derived cell lines. Large numbers of normal individuals and patients with benign diseases need to be tested in standardized cytotoxicity assays, to determine accurately the incidence of "false positive" reactions.

The leukocyte migration assay is thought to be a good in vitro correlate of delayed hypersensitivity skin reactions. Reactivity to common tumor associated antigens has been detected in patients with breast cancer, malignant melanoma, lymphoma and leukemia. With this in vitro assay, tests with tumor extracts would not pose the safety problem mentioned for the in vivo skin tests.

Soluble extracts of autologous and allogeneic tumor cells have recently been reported to stimulate the lymphocytes of cancer patients. These workers have postulated that these soluble antigens could only stimulate lymphocytes of immune individuals, in contrast to the ability of antigens on intact cells to elicit a primary immune response in vitro. Since some tumor extracts, particularly from breast cancer, have produced stimulation of normal lymphocytes, it is unlikely that this assay will be applicable to diagnostic problems.

Detection of antibodies to tumor associated antigens is potentially a very sensitive and logistically simple procedure for immunodiagnosis. Unfortunately, there have been relatively few advances in this area. Antibodies to common antigens on some tumors, e.g., melanoma and osteosarcoma, have been described. However, a considerable number of normal donors gave positive reactions, and the specificity of the detected antigens has not been well characterized. Morton and associates observed a good correlation between clinical status and antibody titers, and serial antibody determinations may prove useful for prognosis and for monitoring therapy.

Humoral factors, which can either inhibit (blocking factors) or facilitate (unblocking factors) cell-mediated immune reactions, may also be useful in immunodiagnosis. Most of the studies of the Hellströms and their associates indicated a close correlation between clinical status and the presence of these factors. Blocking factors were usually found in the sera of patients with detectable tumors. Unblocking factors have been described in the sera of tumor-free patients. However, these correlations have not been complete. Some patients with growing tumors have not had detectable blocking factors, and blocking factors have been found in the sera of some tumor-free patients. Further, careful correlation of serial serum specimens and clinical state is needed to evaluate adequately the usefulness of these factors for prognosis and for monitoring of therapy.
Conclusion
A wide variety of immunological approaches to the diagnosis of human cancer has been described. On the basis of present information, several of these seem particularly promising. However, none of these assays has been adequately evaluated for its possible role in the diagnostic armamentarium of the practicing clinician. Further tests of these procedures are needed in the actual clinical settings in which the assays might be used. Good figures are needed on the accuracy of each test, or of the combination of several tests, for the various practical applications. These studies will be difficult to design and perform, but they should establish the value of immunoLOGY in the diagnosis of cancer.

References
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63. Yalow, R.: Personal communication.