Classification of the Bladder Cancer Patient Based on in Vitro Measurements of the Immune Response

Paul H. Lange, Thomas R. Hakala, and Elwin E. Fraley

Department of Urologic Surgery, University of Minnesota College of Health Sciences, Minneapolis 55455 [P. H. L.], Division of Urology, Veterans Administration Hospital, Minneapolis, Minnesota 55417 [P. H. L.; E. E. F.], and the Department of Surgery, Division of Urology, University of Pittsburgh, Pittsburgh, Pennsylvania 15261 [T. R. H.]

Summary

In vitro measurements of the immune response in patients with cancer can be divided into those that estimate nonspecific and those that estimate tumor-specific immune responses. Contained herein is a review of these measurements, especially as they relate to studies that have been reported in patients with transitional cell carcinoma (TCC). In vitro tumor-specific immunity has been extensively examined in TCC using the lymphocyte-mediated microcytotoxicity assay, but subsequent observations on this assay have seriously jeopardized the validity of those early findings. Recent modifications of this assay have permitted longitudinal studies of lymphocyte cytotoxicity in TCC patients, and clinical correlations suggest that this modified assay may detect important immunological events. To date, however, a clinically useful classification of the TCC patient based on in vitro measurement of immune responses has not been achieved, although many promising areas still require investigation.

Even though tumor immunologists have yet to develop techniques that reliably predict the prognosis in human cancer, assessment of the immune response in patients with cancer including TCC have provided clinicians with some information that has had both descriptive and prognostic value. These measurements can be divided into 2 categories, those that describe the immune response in general (nonspecific immune responses) and those that attempt to discover the immune responses that are specific against TAA. The purpose of this paper is to review those studies that have attempted to measure TCC-related immune responses in vitro.

Nonspecific Measurements of Immune Function (Table 1)

There is increasing awareness that lymphocytes can be categorized into many subpopulations, each with unique morphological and functional characteristics. Recently, the methodologies for detecting and separating these subpopulations have been developed in man, but their application to the study of the patient with cancer is just beginning (32). Rosetting and immunofluorescence are the techniques that have been used to differentiate these lymphocyte subpopulations including T-cells and various non-T-cells, such as Fc-receptor-containing lymphocytes, complement-receptor-containing lymphocytes, immunoglobulin-possessing lymphocytes, and other cells with various combinations of these receptors or none at all (47). In cancer patients with types other than TCC, a depression in both T-cells and immunoglobulin-possessing cells has been associated with advanced malignancy and with poor prognosis, while the T-cell/B-cell ratio is changed after either chemotherapy or radiation therapy (32). Lymphocyte subtypes have been studied very little in TCC patients; however, Catalona et al. (6) presented data showing that T-cell levels are depressed and that such depression is related to tumor stage. Elhilali et al. (9) reported depressed T-cell and complement-receptor cell numbers in urological patients as a group, but they did not tabulate the data separately for each cancer type.

The functional activity of T-lymphocytes is often assessed by their ability to undergo blastogenesis in response to contact with tumor-unrelated stimulators such as phytohemagglutinin or allogeneic cells. In TCC, several studies have demonstrated that depressed blastogenic responses are usually seen in advanced disease (7, 39). Similar findings are reported in other cancers, but differing methodologies and variable results make uncertain their predictive value (29).

Another functional type of lymphocyte, the suppressor T-cell, has received much experimental attention in animals (15), but similar studies in man are just beginning. In the only TCC-related work in this area, Herr et al. (31) suggested that the lymph nodes of some TCC patients contain lymphocytes that have suppressive activity in blastogenesis assays. Measurements of non-T-lymphocyte functions include determinations for the presence and levels of various serum antibodies and the assessment of ADCC. Measurement of antibody levels has been performed in a variety of solid-tumor patients including TCC, but results have shown little difference from age-matched controls (29). Recently, the estimation of the nonspecific functional capacity of Fc-
receptor-containing lymphocytes (the effector cell in ADCC reactions) has received increasing attention in clinical studies because of evidence suggesting a role of ADCC in tumor immunology (8). However, preliminary studies on the ADCC function of lymphocytes from urological cancer patients show no difference from age-matched controls (9).

Macrophages (blood monocytes, tissue macrophages) subserve a role in cell-mediated responses against tumors (33). The ability of these cells to respond in vitro to antigens or cell products unrelated to TAA allow an estimation of “non-tumor-specific” macrophage function. Such determinations include blood-monocyte levels, assays of chemotaxis and migration inhibition, and tests of phagocytosis function and cytoidal activity (14, 32, 48). Some of these tests have revealed macrophage function to be deficient in a variety of advanced malignant diseases. Accordingly, Hausman and Brosman (25) showed that monocytes in 39 TCC patients were defective in their response to nonspecific chemotactic factors. However, problems with methodology make it impossible to evaluate the usefulness of these nonspecific macrophage assays at present.

Several serum factors effecting immune responsiveness have been measured in cancer patients. An immunoregulatory peptide found in the α-globulin serum fraction is present in extremely small amounts in normal sera and causes nonspecific depression of several T-cell functions. This peptide is elevated in the sera of patients with a variety of cancers (16, 40). McLaughlin and Brooks (39) reported the existence of an α-globulin factor in the serum of TCC patients which, they postulated, decreased the responsiveness of the patient’s lymphocytes in blastogenesis assays. Serum complement and interferon levels have also been suggested as important nonspecific immune parameters (32, 52).

In summary, the utility of estimating the nonspecific immune response in TCC patients is uncertain for several reasons. One reason is that it is clear that many of the tests must be measured simultaneously for differences between cancer and normal patients to be appreciated. Another reason is that one must be careful to distinguish those changes that are related to advanced disease and debilitation or to various cancer therapies from those changes that might be causally associated with the cancer itself. Finally, there are significant technical problems with many of these assays which cause unexplained variation in test results even among normal controls; standardization of these assays is therefore necessary.

**Tumor-specific Measurements (Table 1)**

Major efforts in the field of human tumor immunology have been devoted to the development of assays that measure specific immunity to tumors. Initially, the aim was to find tumor-specific antibodies in the cancer patients’ sera. Among the assays used to detect these antibodies were microcytotoxicity, immunofluorescence, immunofluorescence, and complement fixation. These methods revealed the presence of circulating antitumor antibodies in a variety of human tumors including Burkitt’s lymphoma, melanoma, and sarcoma (18). Complement-dependent antibodies that were cytotoxic to TCC cells in vitro were found in the sera of a small number of TCC patients (19). Using indirect immunofluorescence techniques, we detected a humoral antibody directed specifically against the TCC cell line 253J (12). Although this antibody was found in the sera of patients with and without TCC, its titer was significantly higher in the sera of TCC patients than in the sera of normal donors, patients with other kinds of tumor, or patients with nonmalignant urinary tract disease (11). However, the importance of antibody alone as a host defense against cancer is in doubt, since tumor immunity in animals can seldom be conveyed by serum but can be commonly transferred by cell suspension transfusion.

The demonstration of macrophage participation in tumor-specific responses in animals has stimulated similar investigations in man (13, 33, 50). Two assays that have been used to assess macrophage-like antitumor activity are the leukocyte migratory inhibition assay and the leukocyte adherence inhibition test. In these assays, material extracted from tumor cells is used as antigen. Specific inhibition of leukocyte migration has been detected in breast and intestinal cancer, malignant melanoma, and lymphoma. Unresolved technical problems and lack of specificity have limited the usefulness of this assay (29). The leukocyte adherence inhibition assay is simple to perform and may more reliably...
detect tumor-specific immunity. Specific inhibition of monocyte adherence to glass in the presence of tumor antigen has been described in the sera of patients with breast and colon carcinomas and with malignant melanoma (17, 29, 38). No studies of macrophage antitumor activity have been reported in TCC patients.

The importance of lymphocyte participation has been clearly shown in animal immunity to tumors; therefore, it is not surprising that the role of lymphocytes has been examined extensively in human cancer. Efforts to detect lymphocyte blastogenic responses to tumor cells or extracts have been numerous, but none has been developed to the degree of accepted clinical usefulness (29). The assay most frequently used to detect lymphocyte-mediated tumor immunity has been the LMC assay. This assay is performed by combining known numbers of target cells derived from tumors with differing amounts of lymphocytes. The damaging effect of lymphocytes against these tumor cells is then estimated, and cell-mediated immunity is calculated by comparing target-cell survival after exposure to control lymphocytes (or media alone) with target-cell survival after exposure to test lymphocytes. Comparison with similar tests using target cells derived from normal tissue or from other tumors is required to establish the tumor specificity of the cytotoxic reaction.

The LMC assay was applied to many human tumors and almost uniformly revealed that, unlike chemical or viral tumors in animals, human TAA usually were “tissue specific;” that is, lymphocytes from a cancer patient would kill his own tumor cells or cells derived from other tumors of the same histological type but would not kill tumor cells of different histological types. Moreover, on examination of the interaction of sera with lymphocytes, substances (probably antigen or antigen-antibody complexes) were found that would block the cytotoxic effect of lymphocytes in a tumor-specific fashion (27).

Transitional cell cancer has been the most extensively investigated of all the genitourinary tumors using LMC assays (1, 2, 4, 10, 21, 41, 42). Undoubtedly, this is because TCC is a common tumor and because several long-term cell lines derived from these tumors are available. The following is a summary of these early LMC studies: (a) approximately 50 to 80% of TCC patients have LMC to TCC target cells that usually is “tissue specific;” (b) the incidence of this LMC is greater in low-stage than in high-stage TCC; and (c) as described by O'Toole et al. (42), clinical destruction of TCC cells with possible dissemination of TAA (e.g., after irradiation) leads to increased LMC against TCC; removal of TCC cells (e.g., by surgery) leads to decreased LMC. Reappearance of LMC after excisional therapy is associated with tumor recurrence.

The role of serum factors capable of blocking LMC in TCC patients is unresolved. Although Bubenik et al. (4) and O'Boyle et al. (41) reported finding blocking factors in several TCC sera, these observations have not been confirmed by other investigators.

During our attempts to detect blocking factors, we encountered occasional serum factors that enhanced the in vitro killing of TCC cells by lymphocytes. Details of these studies have been reported elsewhere (20, 22). Briefly, the factor(s) was found: (a) to be an IgG that induced lymphocytes to become cytotoxic against TCC cells (4 different lines) but not against other kinds of tumor or normal cells (11 different lines); (b) to require contact with TCC antigen(s) before it could induce lymphocyte cytotoxicity; and (c) to induce, at high dilutions, cytotoxicity in lymphocytes from TCC patients more often (67%) than in lymphocytes from other patients (14%).

Our evidence for a TCC-specific LDA added support to the hypothesis that TCC has TAA. Other reports have also presented evidence for tumor-associated LDA in some sera from other kinds of tumor patients (26, 36, 45, 51, 55). However, all these studies, including our own, are inconclusive; the possibility that these LDA-like activities are directed against histocompatibility, blood group, or other tumor-unrelated target cell antigens has not been completely resolved. Although the LDA-like serum factor is uncommon in TCC patients, the search to find more of these sera is continuing, because the availability of such a factor could reveal much about the pattern of tumor-immune responses in TCC and possibly help further to classify the TCC patient immunologically.

Despite an impressive number of reports in support of tumor-specific cytotoxicity as demonstrated by the classical LMC assay, the very relevance of in vitro LMC assays to studies of human tumor immunity is now uncertain on the basis of the following, more recent observations (5, 28, 30, 44): (a) lymphocytes from normal patients frequently are cytotoxic to tumor cells, including TCC, occasionally in a pattern suggesting tissue specificity; (b) as the number of different types of tumor target cells tested increases, lymphocytes from many cancer patients, including TCC, are often found not to have histological tumor-specific reactivity but, instead, nonselective cytotoxicity or cytotoxicity directed to target cells derived from other types of tumors; (c) the relationship of the lymphocyte/target-cell ratio to target cell destruction is nonlinear; therefore, many different lymphocyte concentrations must be tested to assess the cytotoxic behavior of a particular lymphocyte population accurately; and (d) although differing methodologies (e.g., lymphocyte separations, selection of target cells, counting by isotopes or visually, etc.) influence results, these differences do not explain the nonselective or tumor-unrelated cytotoxicities encountered.

In an effort to overcome some of these problems, we have modified the classical LMC assay so that the results are proportional to lymphocyte activity. The details of this modified assay have been reported elsewhere (24). Briefly, each lymphocyte population is tested against many different human tumor cell lines using 7 different concentrations of lymphocytes against each cell line. The number of lymphocytes needed to kill 50% of the target cells compared to media controls is calculated and then converted into a logarithmic number (called the LLD<sub>50</sub>) such that the larger the number the greater is the cytotoxicity. This modified assay, called the lymphocyte titration assay, is very reproducible over time and allows the serial estimation of lymphocyte cytotoxicity during the clinical course of TCC patients.

A LMC assay that is able to measure patient LMC longitudinal could produce important data because: (a) multiple antigenic differences obviously exist between human lymphocytes and what are usually allogeneic tumor cells; and
(b) the detection of human tumor-specific immunity against this background of multiple, presumably tumor-unrelated antigenic differences may be possible with serial estimations of LMC; that is, changes in cytotoxicity, if confined only to the appropriate target cell and associated with removal or recurrence of tumor, might reveal immunity directed against TAA.

Using the lymphocyte titration assay, we are estimating the serial lymphocyte cytotoxicity against 4 to 6 different kinds of human tumor target cells during the clinical course of TCC patients. Preliminary results show that some of these patients have apparent tumor-associated, target cell-specific changes in cytotoxicity that coincide with changes in their tumor burden. Although we occasionally find a decline in lymphocyte cytotoxicity against TCC cells, as previously reported (42), we more frequently see both short- and long-term rises in antitumor target cell cytotoxicity following reduction in tumor burden (23). Chart 1 illustrates this observation in 1 such TCC patient who had recurrent noninvasive bladder tumors treated by transurethral resection.

However, a significant number of TCC patients followed by the lymphocyte titration assay failed to show substantial changes in cytotoxicity when their tumor burden changed; it is apparent that this assay measured only 1 aspect of the patient's immune response to his tumor and that other components, such as serum factors, require simultaneous evaluation. Despite these shortcomings, the lymphocyte titration assay may help pinpoint those immunological events in the clinical course of TCC patients that are significant and worthy of more intensified investigation.

Our preliminary experience indicates that the lymphocyte titration assay also may be useful in following patients with advanced TCC and renal cell carcinoma who are receiving BCG immunotherapy (37). In 11 patients followed to clinical deterioration or for at least 18 months, a change in the cytotoxic level appears to be related to clinical outcome; that is, almost all patients (4 of 5) who exhibited an increase in their LLD50, with BCG therapy have had a favorable course (either remission or stable disease), whereas those 6 patients with no LLD50 response to BCG have had a progressive downhill course. In a majority of cases, the cytotoxic patterns seen with BCG are "nonspecific" (e.g., cytotoxic reactivity of all target cells goes up after therapy) although, occasionally, we have seen tumor-associated, target cell-specific changes (e.g., an increase in LLD50 in a TCC patient only to TCC cells). However, these correlations are not perfect; 1 of our renal cell carcinoma patients had a dramatic remission in his pulmonary metastases with an actual decline in cytotoxic reactivity (Chart 2). Thus the lymphocyte titration assay appears to be a reliable, but not perfect, predictor of clinical events related to immunotherapy and is sometimes nonspecific. Parenthetically, several groups (34, 35), including our own (unpublished data), have determined that the cytotoxic cell of the LMC assay is a subpopulation of non-T lymphocytes, probably a "K" or "null" cell; also, several reports of results obtained with different methods have suggested that BCG specifically stimulates these cells (46, 54).

The Future

It is not yet possible to classify or monitor the TCC patient based on available in vitro immune measurements. Furthermore, earlier findings concerning the presence and behavior of TCC-specific immunity must now be reexamined. However, one should not be pessimistic about the future role of immunological monitoring in the TCC patient, because the studies to date have produced meaningful immunological information and suggest many new avenues for investigation.
Classification by in Vitro Immune Response

For example, results from our laboratory (unpublished data) and others (3, 53) indicate that TCC patients as a group do have higher cytotoxic levels to TCC cells, but not to other types of cells, than do other patient groups. Thus, cancer-associated, target cell-specific immunity may be superimposed upon a background of other nonspecific or specific, but cancer-unassociated, reactivities. The LMC assay, therefore, might be further modified to focus more sharply on tumor-associated cytotoxicity, as for example: (a) by testing large numbers of target cells and lymphocytes using sophisticated statistical methods to distinguish selective from nonselective cytotoxicity (49); (b) by determining the LMC of separate lymphocyte subpopulations in order to distinguish normal or nonselective cytotoxicity (e.g., K-cell cytotoxicity) from possible disease-related cytotoxicity (34); (c) by removing tumor-unassociated cytotoxicity by inhibition with tissue extracts or by elimination of certain immune cells through absorption techniques; (d) by increasing the use of autochthonous reactions in these cytotoxic assays (our ability to establish TCC in culture is improving, and recent evidence suggests that the effector cell type in such reactions is a T-cell rather than a non-T-cell, as is the case with allogeneic TCC reactions) (43); and (e) by improved standardization of the LMC assay, as, for example, with the use of frozen lymphocytes and fully characterized tissue culture cell lines.

In conclusion, the immunological evaluation of TCC patients should not concentrate on 1 test. Many parameters, both tumor specific and nonspecific, must be correlated with each other and with the clinical course for the maximum development of knowledge. Also, the isolation of purified TCC TAA, a task that is becoming increasingly feasible, may provide important opportunities both for immunodiagnosis and for more accurate evaluation of the immune response to TCC. Finally, the use of immune techniques not only should focus on the immune response of the TCC patient but also should concentrate on the tumor itself. Determining the antigenic profile of the TCC cell, both with regard to normal and unique antigens, may provide valuable additional information about patient prognosis.

References


7. Catalona, W. J., Tarpley, J. L., Chretien, P. B., and Castle, J. R. Lympho-


Classification of the Bladder Cancer Patient Based on *in Vitro* Measurements of the Immune Response

Paul H. Lange, Thomas R. Hakala and Elwin E. Fraley


**Updated version**
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/37/8_Part_2/2885

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.