Immunological Responsiveness in Patients with Bladder Cancer

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Summary

Delayed cutaneous hypersensitivity tests, especially skin tests with dinitrochlorobenzene, are impaired increasingly as the amount of tumor increases. Recall antigens are less sensitive indicators of disease. Therapy, especially radiotherapy, also depresses cell-mediated immunity. Removal of tumor, however, allows these tests to return to normal.

Dinitrochlorobenzene skin testing can contribute significantly to prognostic evaluation. An important facet of the tumor-host relationship is measured, and this reflects factors that are independent of tumor staging. Combination of tumor staging and dinitrochlorobenzene-delayed hypersensitivity testing can provide a strong indication of the clinical course, especially for the year following initial treatment of invasive or metastatic transitional cell carcinoma of the bladder.

Introduction

Host immune control is an important factor in the natural history of cancer (2). Furthermore, impaired immunocompetence, especially impaired cell-mediated immunity, is often found in cancer patients (9, 16). Much of the data on immune incompetence in cancer patients was obtained with skin tests. The in vivo tests have included evaluation of primary delayed hypersensitivity responses and evaluation of delayed hypersensitivity response to recall antigens (9, 10, 18). For the former test, DNCB3 or keyhole limpet hemocyanin have been most used. For the later test, antigens such as mumps, Candida, streptokinase-streptodornase, dermatophytin, and tuberculin are commonly used.

Delayed hypersensitivity testing by intradermal injection or application of appropriate recall antigens is impractical because of the variety in intensity and range of prior exposure that will occur in a patient population. Also, recording a response to 2 or more recall antigens as positive and to 0 or 1 antigen as negative is arbitrary.

Primary induction of contact (delayed type) hypersensitivity with DNCB and subsequent challenge with the same agent requires full function of the cellular immune circuit for a positive response. T-cell function is essential. This test has proved to be quite useful in relating immune changes to tumor extent (10). The test system, however, is arbitrary, and it may not be "set" properly for assessing some patients. Furthermore, the DNCB test is only partially quantitative. Serial testing also can present problems due to accumulated antigen effects. Finally, many immunological components are probably required for effective induction and subsequent response. Although a complex sequence of reactions is required for this test to become positive, it may be this very complexity that is responsible for the great sensitivity of this skin test. In vitro detection of cellular reactivity to DNCB after sensitization has been reported, but experience in many laboratories indicates difficulty in conducting reproducible in vitro tests of DNCB reactivity.

Croton oil induces a nonimmune inflammatory response, and this response has been noted to be impaired in patients with neoplastic disease (17, 22). There is disagreement about whether this does (17) or does not parallel (22) the reduction in DNCB response. Our data confirm the depressed response in patients with bladder cancer and indicate that it does not absolutely parallel the DNCB response change. This test too is difficult to quantify.

Patients with normal levels of cell-mediated immune function have a good prognosis whereas patients with deficient function have a poor prognosis (9, 15). This applies in acute leukemia in terms of remission rates, remission duration, and survival subsequent to testing (13). Most important, a sudden decline in immunocompetence in a previously normal patient in remission heralds relapse. The phenomenon of impaired immune competence is reported in all forms of cancer. Furthermore, this relationship between immune function and prognosis is one of the most important prognostic features of cancer, but it is modified by all forms of cancer treatment.

Immunodeficiency is induced by the conventional modes of cancer treatment, particularly radiotherapy and chemotherapy. These include immunosuppressive effects of anesthetic agents (1), immunosuppression associated with stress of surgery (21), immunosuppression induced by radiotherapy (24), and chemical immunosuppression (12). The latter is quite complex because, although continuous chemotherapy is definitely immunosuppressive, intermittent chemotherapy may not be, especially if the number of courses given is relatively few, if the interval between courses is relatively long, and if certain classes of drugs, particularly alkylating agents and nitrosoureas, are avoided (10). In addition, after the end of a short course of chemotherapy, immunological rebound may occur, resulting in a heightened immune response above the baseline level in some individuals (18), and the latter often have an improved prognosis. Immune modulation characterized by the suppression of antibody production without suppression of cell-mediated immunity may occur (11). Intermittent chemo-
therapy tends to spare cell-mediated immunity for a while after its inception, but patients on treatment for several years receiving combination chemotherapy at approximately monthly intervals often manifest relatively severe immunodeficiency (23). Not only can such patients relapse, but they also suffer from a markedly increased incidence of opportunistic infections, normally resisted through mechanisms of cell-mediated immunity (3). However, even in this group, immunodeficient as a whole, those with relatively better immunocompetence have a better prognosis than those with relatively poor immunocompetence.

Bladder Cancer

Impaired immunological reactivity has been reported in patients with transitional cell carcinoma. Reduced delayed hypersensitivity skin test responses to keyhole limpet hemocyanin (primary immunization) and common recall antigens (secondary response) were reported by Olsson et al. (20). Impaired delayed hypersensitivity responses in a primary immune response to DNCB were observed in several clinics (4, 7, 19), particularly in patients with extensive tumor. Impaired delayed hypersensitivity skin tests clearly have poor prognostic significance (5, 7). Catalona et al. (5) found that 60% of 19 patients with negative DNCB tests died within 1 year whereas all 9 patients with positive DNCB tests remained alive. Decenzo et al. (7) evaluated patients in stages A, B, C, and D with a 2-year follow-up and found no correlation of DNFB skin test response with disease progression other than that predictable from tumor staging.

UCLA Studies

Our initial hypothesis was that a measurement of immunological or inflammatory parameters should give us a good index of important host defense systems that would enable us to assess the effects of disease, conventional therapy, and immunotherapy. An array of immunological tests designed to assess specific functions has been developed, and they are still in the process of testing and new development. The results to date have been particularly interesting in relation to the delayed hypersensitivity skin tests (8).

Skin testing was undertaken to assess the response to primary immunization to the chemical dye and for assessment of secondary response by use of recall antigens to mumps, Candida albicans (monilia), purified protein derivative of tuberculin, and streptokinase-streptodornase. Finally, inflammatory response was assessed by the use of croton oil, which is itself a primary irritant and does not involve an immunological response.

DNCB proved to be the most useful of these tests. The recall antigen tests were roughly similar to the findings observed with the DNCB tests but did not show such good correlation with the extent of disease or prognosis. Curiously enough, none of the 4 antigens was of particular value except streptokinase-streptodornase in certain situations. Croton oil provided correlation with disease extent that was roughly comparable to that of DNCB. There was no exact correspondence, however, between the patients who were negative for the DNCB and those who were negative for the croton oil test. A provocative possibility is that the cancer in some cases was affecting the inflammatory response more markedly than the immune response but that the opposite was true in other patients. Further exploration of this interesting discrepancy has been deferred in order to concentrate on the DNCB studies.

Effects of Transitional Cell Carcinoma

Disease was assessed as being localized, Stage A (Group I); invasive, Stages B and C (Group II); and metastatic, Stage D (Group III). The results of DNCB, recall antigens, and croton oil testing in bladder cancer are summarized in Chart 1 and compared with renal and prostate cancer of similar stages. The presence of superficial tumor caused impaired (negative) DNCB tests in about 45% of the patients. When the cancer became invasive (Group II), 60% of the patients were unresponsive, and metastatic cancer raised this to 75% of unresponsiveness. This progression was statistically significant with a p value less than 0.005. A similar pattern was observed with croton oil. Recall antigens did not provide such clear distinctions.

A good bit of the depression of skin testing could be attributed directly to the tumor. Removal of the tumor by surgery increased positive reactions to DNCB in a group of patients from 22 to 44%, which was statistically significant (Table 1). Furthermore, if the patients were tumor free for a period of 24 months, the percentage of patients with DNCB-positive tests increased even further (Table 2).

Correspondingly, patients who were originally thought to have localized disease but later developed metastatic disease had a very poor response to DNCB, indicating either
Effect of bladder tumor reduction by surgery or surgery and radiation

| Tumor present (91\textsuperscript{a}) | 22 |
| Tumor removed (85\textsuperscript{b}) | 44 |

\(p < 0.005\)

\textsuperscript{a} Number of patients.
\textsuperscript{b} Treatment 4 or more months earlier.

Table 1
Effect of bladder tumor reduction by surgery or surgery and radiation

Table 2
Skin test results in patients with localized cancer

<table>
<thead>
<tr>
<th></th>
<th>With cancer\textsuperscript{a}</th>
<th>Cancer free\textsuperscript{b} (early)</th>
<th>Cancer free\textsuperscript{c} (late)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNCB</td>
<td>27</td>
<td>53</td>
<td>68</td>
</tr>
<tr>
<td>Recall antigens</td>
<td>36</td>
<td>59</td>
<td>68</td>
</tr>
<tr>
<td>Croton oil</td>
<td>50</td>
<td>71</td>
<td>87</td>
</tr>
</tbody>
</table>

\textsuperscript{a} \(n = 48\).
\textsuperscript{b} \(n = 47\).
\textsuperscript{c} \(n = 45\).

Immunological Responsiveness

Radiation Therapy Effects

Not only does the presence of neoplasm affect the delayed hypersensitivity response, but also radiation therapy can change the test results. Our observations indicate a marked change by radiotherapy when tests are carried out sequentially during the year following radiotherapy (Table 3). DNCB values become markedly depressed. Croton oil response, however, changes less markedly in the same group of patients. This is in accord with evidence from many studies that the lymphoid cells are particularly susceptible to radiation damage, whereas the monocytes and other cells involved in an inflammatory response are less severely damaged, if at all.

Prognosis

Patients with Stage D (Group III) transitional cell carcinoma, \(i.e.,\) those with metastatic disease, show markedly different survival, depending upon whether they are DNCB negative or positive when first tested (Chart 2). In our series to date, approximately 72\% of the patients that were DNCB negative died within 1 year of testing, whereas none of the 5 patients who were DNCB positive died.

Patients with invasive cancer in Group II have a better survival record but show a trend similar to patients who are DNCB positive living longer as a group than those who are DNCB negative (Chart 3).

We feel that these data have many ramifications, not only in clinical practice but also in undertaking trials of new forms of therapy. Clearly, a comparison between 2 treatment groups with unequal percentages of patients who were DNCB positive and DNCB negative would be out of balance. The therapeutic group with the larger number of DNCB-positive patients would be expected to have a better performance record. This consideration would apply whether the therapeutic trials were those involving chemotherapy or immunotherapy.

There are a number of interesting biological questions remaining; \(i.e.,\) why do patients who are in Group II and yet DNCB negative do so well? Approximately one-fourth of the patients die within 1 year, but then the others who have lived 1 year continue to do very well. In the Group 3 (Stage D), a group of 25 or 30\% of the patients will do well, even though they were DNCB negative. These patients survive more than 1 year and continue to live without lethal progression of their disease.

It is not surprising to find that there are these groups. The response of the host to neoplasm is complex, and it would be too much to expect that a single test would measure all of the relevant parameters. Perhaps the DNCB test (which involves a complex response including the afferent, central, efferent, and amplification elements of delayed hypersensitive response) will come closer to assessing general overall competence than do more refined techniques which assess single components of host defense, such as individual cell functions.

Table 3
Effect of radiotherapy on delayed hypersensitivity and inflammatory response

<table>
<thead>
<tr>
<th></th>
<th>DNCB</th>
<th>Croton oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preradiotherapy</td>
<td>38</td>
<td>65</td>
</tr>
<tr>
<td>Postradiotherapy</td>
<td>96</td>
<td>76</td>
</tr>
</tbody>
</table>

\(p < 0.005\) \((p > 0.1)\)

Chart 2. Prognostic relevance of DNCB skin tests in patients with metastatic bladder cancer (Group III or Stage D). Each symbol represents a patient surviving for the indicated time period. Patients not represented by symbols are represented by the stepwise drops indicating the deaths of 1, 2, or 3 patients, or those who have survived beyond 20 months.
Although a DNCB test may be somewhat arbitrary in its application, our experience indicates that certain challenge doses are more valuable than others. Dr. Brosman and Dr. Dorey found that patients who had been sensitized with 2000 μg and then challenged later with 25, 50, and 100 μg gave most valuable data from a prognostic standpoint, if the responses to a 25-μg challenge dose alone (plus inflammatory response to DNCB sensitization) were assessed. These are promising leads. They give evidence that the delayed hypersensitivity reactions are either important components of host defense against tumors or independently reflect the extent of tumor biological activity.

Acknowledgments

The observations benefited greatly from the support of the faculty in the Division of Urology at UCLA Medical Center and the Department of Surgery at Harbor General Hospital. Also Dr. Mustafa Elhilali, when he was a visiting professor at UCLA, contributed a great deal to the initial correlations of clinical observations with immunological parameters. Dr. Robert Elashoff has provided essential guidance in the biostatistical evaluation of these findings. The authors wish in particular to thank Candy Vescera and Pamela Collins who are essential participants in these efforts.

References


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