Delayed Cutaneous Hypersensitivity to Autologous Tumor Cells in Colorectal Cancer Patients Immunized with an Autologous Tumor Cell: Bacillus Calmette-Guérin Vaccine


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carcinoma model were used as the basis of a randomized, ABSTRACT

malignant tumor 

sponses. The qualitative and quantitative differences in DCH responses of immunized patients to autologous normal mucosa, used as a normal tissue control, did not increase significantly. Furthermore, no significant DCH responses against autologous tumor or mucosa cells were detected in a group of nonimmunized control patients. The induced DCH responses were not correlated with other factors, such as the presence of bacteria in the cell preparation or the protein concentration of the cell preparations. The qualitative and quantitative differences in DCH responses to tumor cells and to normal mucosa cells suggest that the immunizations are targeted mainly to tumor-associated antigens with tissue-associated antigens playing a secondary role.

INTRODUCTION

Active specific immunotherapy protocols are based upon the presumed presence of tumor-specific or tumor-associated antigens capable of eliciting an immunological response. There is now extensive evidence that many human tumors have tumor-associated antigens; assessment of specificity of these antigens is difficult (10). Studies using monoclonal antibody technology, however, are presently adding considerable data to this subject (15). The question arises, if tumor-specific antigens do exist, why do patients with malignant tumors have an inadequate immunological response to the tumors? An even more important question is, can tumor cells be altered in such a way as to increase their immunogenicity, thereby making them useful for active specific immunotherapy?

Most studies of active specific immunotherapy in humans have yielded marginal or negative results, engendering the pessimistic attitude held by many clinicians toward this approach to therapy (7, 19). These negative past trials should not cause us to abandon our attempts to manipulate the immune system in favor of the host. Certainly, in diseases such as colorectal cancer and most of the other common solid tumors on which current adjuvant therapies have little or no beneficial effect, our efforts to gain a better understanding of the biology of cancer and tumor immunology to guide us in the development of better multimodal treatment of solid tumor must be increased.

A reappraisal of the past failures in the light of current studies in experimental animal models may clarify the mechanisms and suggest new immunological approaches. Recently, the procedures of active specific immunotherapy with tumor cell:BCG vaccines were reevaluated in a model system using the syngeneic line 10 hepatocarcinoma in inbred strain 2 guinea pigs. Immunotherapy in this model is not successful unless certain conditions are met. (a) The tumor cells in the vaccine must be syngeneic. There is no cross-protection from the antigenically distinct line 1 hepatocarcinoma, even though organ site and mode of tumor induction are the same. (b) At least \( 10^7 \) metabolically active cells must be present in the vaccine. Cells killed or damaged by dissociation or cryopreservation procedures are not effective. (c) The correct amount of appropriate adjuvant must be admixed with the tumor cells. At least \( 10^7 \) but not more than \( 10^8 \) viable BCG antigens are required. (d) Vaccination schedule is important. At least 2 and preferably 3 vaccines administered at weekly intervals are effective (6, 8, 9, 14, 16, 21, 22).

From our studies, we have concluded that the failure of some previous experimental and/or clinical active specific immunotherapy protocols using adjuvants and autologous tumor cell vaccines may have been due to the low viability of the tumor cells. Such cells disintegrate rapidly in the dermal vaccination site and do not provide the necessary sustained antigenic stimulus to the regional lymph node.

In this guinea pig model, as in other models, the success of therapy is inversely proportional to the number, size, and location of metastases present at the time of treatment. Given the variability of clinical presentations, the lack of predictability of response (decreased tumor recurrence) in clinical trials of immunotherapy as translated from animal models is not surprising. Our experimental models cannot be considered analogous to clinical cancer. The experimental injection of tumor cells in any number and in any site is strikingly artificial, since it represents the introduction of a defined tumor load at a single location in an otherwise normal animal. Nonetheless, some of the biological principles of host-tumor interaction revealed in these experimen-

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tal models may be analogous to those involved in naturally occurring cancers in humans. Clinical trials are the only way to test whether these analogies are valid.

Over the past 3 years, we have translated the principles and procedures of active specific immunotherapy as learned in the guinea pig hepatocarcinoma model to a randomized, controlled trial of active specific immunotherapy in patients with colorectal cancer. Our dual objectives were (a) to determine whether active specific immunotherapy could enhance the reactions to colorectal cancer patients to their autologous tumor cells as measured by DCH responses and (b) to determine whether this therapy could prolong the survival of colorectal cancer patients. In this paper, we describe results relevant to the first objective.

MATERIALS AND METHODS

Patient Selection and Randomization. Candidates for our study were patients with colorectal cancer who (a) had no previous history of cancer, (b) had received no prior chemotherapy or radiotherapy, and (c) were in suitable medical condition to comply with the outpatient treatment protocol. Resection of the tumors was performed at the following Baltimore, MD, hospitals: Johns Hopkins; Loch Raven Veterans'; Baltimore City; St. Agnes; and Greater Baltimore Medical Center. The pathological stage of the tumors was assessed according to the Astler-Coller modification of Dukes' system. Patients with tumor extending through the bowel wall (Stage B2), positive lymph nodes (Stages C1 and C2), or metastatic disease (Stage D) were eligible for the trial. Within these classifications, patients were randomly selected for participation in treatment and non-treatment groups. Randomization cards were generated by computer by Dr. R. M. Simon at the Biometrics Branch, National Cancer Institute, NIH, and were sequentially drawn from each category at the time (postoperatively) the patient signed an informed consent form.

Within this randomized study, 24 of 25 immunized patients and 11 of 25 nonimmunized control patients were used to determine whether there was increased reaction to their autologous tumor cells, as measured by DCH responses.

Tumors. After surgical resection, the bowel specimen was taken immediately to the hospital pathology department and opened under sterile conditions. All tumor tissue not required for staging and 2 to 3 g of distal normal colon mucosa were excised, placed in sterile tubes with HBSS containing 50 μg gentamicin/ml, and carried immediately on ice to the laboratory for processing and freezing.

Dissociation of Solid Tumor and Colon Mucosa. The tissue dissociation procedure of Peters et al. (21) was used under sterile conditions under a laminar flow hood (Chart 1). Tumor and mucosa tissue were rinsed 3 times in the centrifuge tube with HBSS and gentamicin and transferred to a Petri dish on ice. Excraneous tissue was excised with a scalpel, and the tumor was minced into pieces approximately 2 to 3 mm in diameter. The same care was taken with the colon mucosa tissue. Tissue fragments were placed in a 125-ml flask with 20 to 40 ml of 0.14% collagenase type 1 (200 units/ml; Sigma C-0130) and 0.1% DNase type 1 (500 Kunitz units/ml; DNase 1, Sigma D-0876) prewarmed to 37°. Flasks were placed in a 37° water bath with submersible magnetic stirrers (Tri-R, Rockwell Center, NY) at a speed that caused tumbling, but not foaming. After a 30-min incubation, free cells were decanted through 3 layers of sterile medium-wet nylon mesh (166T; Martin Supply Co., Baltimore, MD) into a 50-ml centrifuge tube. The cells were centrifuged at 1200 rpm (250 × g) in a refrigerated centrifuge for 10 min. The supernatant was poured off, and the cells were resuspended in 5 to 10 ml of Dnase (0.1% in HBSS) and held at 37° for 5 to 10 min. The tube was filled with HBSS, washed by centrifugation, resuspended to 15 ml in HBSS, and held on ice. The procedure was repeated until sufficient cells were obtained, usually 3 times for tumors and once for colon mucosa. Cells from the different digests were then pooled and counted, and the cell viability was assessed by the trypan blue exclusion test. Cells were centrifuged for a final wash before cryopreservation.

Bacteriological Testing of Colon Mucosa and Tumor Cell Preparations. Supernatant fluids from the final wash of the enzyme-dissociated cell preparations were submitted to bacteriological evaluation. Bactec vials were inoculated and incubated at 30° under aerobic conditions or 37° under anaerobic conditions and monitored for 10 days. Positive vials were plated to an appropriate medium for isolation and identification of the organisms.

Cryopreservation. The importance of optimal cryopreservation has been described (22). For vaccine preparation, the dissociated tumor cells were adjusted to 4 to 8 × 10¹⁰/ml in HBSS and added in equal volume to chilled 2× freezing medium containing 15% dimethyl sulfoxide and 4% human serum albumin. The final suspensions of 2 to 4 × 10¹⁰ cells/ml were placed in 1.2-ml Nunc freezer vials. For DCH testing of both tumor and mucosa cells, the preparation procedure was the same except that no serum albumin was used. In both cases, before freezing, the Nunc vials were transferred on ice to a Cryo-Med Model 990 biological freezer with a Model 700 controller and a Model 500 temperature recorder for controlled-rate freezing. Care was taken that the temperature of the individual vials, including the monitor vial, was uniform at the beginning of the freezing process. Vials were cooled at a controlled rate of −1° to −4° and then at −5°/min to a final temperature of −80°. The vials were transferred in liquid nitrogen to liquid nitrogen storage.

Clinical Protocol. Patients with tumors of the appropriate pathological stages were randomized either to receive autologous tumor cell/BCG vaccine or to have no further therapy. The Stage D patients all received 4000 rads of pelvic radiation 2 weeks after immunotherapy was completed. The vaccines were started at 4 to 5 weeks after tumor resection to allow the patient sufficient time to recover from immunological suppression induced by anesthesia and surgery. At 3 to 4 weeks after resection, both control and treatment patients were skin tested with standard recall antigens and with graded doses of autologous tumor and mucosa cells (see below). Recall antigens used were: mumps skin test antigen (U.S.P.; Eli Lilly, Indianapolis, IN); Aplisol (tuberculin-purified protein derivative) (Parke-Davis, Detroit, MI); Trichophyton, diluted 1:30 (Center Laboratories, Port Washington, NY); and Candida albicans, diluted 1:100 (Center Laboratories). Injections (0.1 ml) of each antigen were given i.d. into the forearm, and the sites were examined for erythema and induration at 24 and 48 hr.

Treated patients received one i.d. vaccine per week for 2 weeks of 10³ irradiated, autologous tumor cells and 10³ BCG and one vaccination of 10⁷ tumor cells alone in the third week. Fresh-frozen Tice BCG was supplied by Dr. Ray Crispin, University of Illinois Medical Center, Chicago, IL, and was stored at −70°. The first vaccine was placed on the left anterior thigh approximately 10 cm below the groin crease, the second in a comparable location on the right thigh, and the third in the right deltoid area. On the days of the first and second vaccinations, the vial was rapidly thawed in a 37° water bath, and tumor cells were diluted slowly to 15 ml in HBSS. The cells were counted, and cell viability was determined.
with the trypan blue exclusion test. Viability ranged between 70 and 90% with a mean of 80%. The cells were washed once by centrifugation at 1200 rpm and resuspended to 15 ml in HBSS. The suspension was placed on ice and irradiated with 4,020 rads/min for a total of 20,000 rads in a Shepherd Mark I 137cesium irradiator. The volume of the cell suspension was adjusted such that $10^7$ viable tumor cells remained in the tube ($1.3 \times 10^7$ viable cells were included to allow for cell loss in tubes and syringes and for the possibility of approximately 20% misidentification of lymphoid cells). The cells were centrifuged, the supernatant was removed, and $10^7$ viable BCG antigens (as determined by colony-forming units) were added in a volume of 0.1 ml. HBSS was added in sufficient quantity for a final volume of 0.2 ml. The third vaccine was similarly prepared, omitting the BCG.

The vaccine suspension was drawn up through a 20-gauge needle into a 1.0-ml tuberculin syringe. The 20-gauge needle was replaced with a 27-gauge needle for the i.d. injection, and the syringe was placed on ice for transport to the clinic.

The patients were observed closely after each vaccination for erythema and induration at the site of injections, fever, lymphadenopathy, or any adverse reactions. The first 2 vaccination sites ulcerated in all patients after 2 to 3 weeks but always healed within 10 to 12 weeks.

Colon Mucosa and Tumor Cell Skin Test Preparation and Administration. One week before the skin testing, tumor and colon cells frozen in dimethyl sulfoxide:HBSS (without serum albumin) were rapidly thawed in a 37° water bath. Using sterile technique throughout, frozen-thawed cells were diluted slowly to 15 ml in HBSS, counted, and assessed for viability by trypan blue exclusion. The numbers of both viable and nonviable cells were calculated. Cells were washed once, resuspended in HBSS, placed on ice, and irradiated at 4,020 rads/min for a total of 20,000 rads with a Shepherd Mark I 137cesium irradiator. After irradiation, the cells were centrifuged at 1,200 rpm for 10 min and resuspended to a concentration of $5 \times 10^6$/ml ($1 \times 10^8$/0.2 ml). Serial 1:10 dilutions were made to provide $10^5$/0.2 ml and $10^4$/0.2 ml. Portions (0.3 ml) of each dilution were placed into 4 sterile 1.2-ml Nunc vials and frozen at −70° in a Revco freezer.

At the time of the skin testing, the vials were thawed rapidly at 37°, and 0.2 ml of the suspension was drawn up through a 20-gauge needle into a 1.0-ml tuberculin syringe. The 20-gauge needle was replaced with a 27-gauge needle, and the syringe was placed on ice for transport to the clinic. Skin testing was conducted as follows. The anterior thigh was swabbed with 70% alcohol. Colon mucosal cells (105) were injected i.d. in the medial aspect of the thigh about 10 cm below the groin crease. The 105 and 104 dilutions were placed 5 and 10 cm, respectively, below that. The corresponding 3 dilutions of tumor cells were similarly placed on the lateral aspect of the thigh. Sites of injection were marked with indelible ink.

Induration was measured at 24 and 48 hr; a medium ball point pen was placed at a 45° angle to the patient's skin, and the injection site was approached from each of 4 sides until resistance was encountered. The area thus circumscribed was measured in 2 diameters with a caliper. Selected DCH sites were excised at 48 hr for histological analysis.

Skin testing was done at 6 weeks, 6 months, and 1 year after vaccination, using alternate legs for testing. Results were statistically evaluated using a paired t test for differences between groups or means (2 tailed).

**RESULTS**

Reactivity to Standard Recall Antigens. All patients reacted initially to at least one of the standard recall antigens. Two of 29 reacted to *C. albicans*, 26 of 29 reacted to mumps, 16 of 29 reacted to purified protein derivative of tuberculoprotein, and 3 of 29 reacted to *Trichophyton*. There was no significant change in reactivity in the follow-up period, except that all but 2 of the immunized patients converted to purified protein derivative positive.

**DCH to Tumor Cells.** The DCH reaction to $10^6$ autologous tumor cells in 24 immunized and 11 nonimmunized control patients is shown in Table 1. A 48-hr induration measurement of greater than 5 mm was considered positive. Four of 24 patients (17%) had a positive DCH response to $10^6$ tumor cells before the course of immunization. This was not significantly different from the one of 11 patients (9%) that tested positive in the nonimmunized control group. Significantly ($p < 0.01$), all 4 of the initially positive responders and 12 of the negative responders in the immunization group exhibited enhanced DCH reactivity after immunotherapy (67% became positive). Seven of these patients have been tested at 1 year; 3 have maintained the positive response. Only 3 of 16 immunized patients demonstrated a positive DCH response to $10^5$ tumor cells at 6 weeks; none of the patients responded to $10^4$ cells.

**DCH to Colon Mucosa Cells.** The DCH response to injection of $10^6$ autologous mucosa cells in the 2 groups of patients is shown in Table 2. None of the nonimmunized control patients reacted significantly to normal colon mucosa cells. Two of 24 patients (8%) in the treatment group responded positively to normal mucosa cells in the preimmunization testing, and 5 other patients responded positively at 6 weeks or 6 months after immunization (29% positive overall). There was a significant difference ($p < 0.01$) between the postimmunization DCH response to normal mucosa cells (7 of 24 patients) and the increased DCH response to tumor cells (16 of 24 patients) (Table 1).

Chart 2 shows the differences between the reactivity to tumor and mucosa cells at each test period. The mean area of induration at 48 hr was significantly larger at the tumor cell sites at both 6 weeks ($p < 0.03$) and 6 months ($p < 0.01$). Mucosa cell sites demonstrated an insignificant increase in the mean indura-

<table>
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<tr>
<th>Stage</th>
<th>No. of patients</th>
<th>Preimmunization reactivity*</th>
<th>Range (sq mm)</th>
<th>Reactivity (6 wk and/or 6 mo)</th>
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<td>2</td>
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* Reactions were considered positive when the 48-hr induration (the mean of 2 diameters) was more than 5 mm.

Numbers in parentheses, total percentage of patients expressing either pre- or postimmunization reactivity.
Table 2

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<tr>
<th>Stage</th>
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<th>Preimmunization reactivity (sq mm)</th>
<th>Reactivity (6 wk and/or 6 mo)</th>
<th>Range (sq mm)</th>
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<td>1</td>
<td>63.6</td>
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<tr>
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<td></td>
<td>24</td>
<td>2 (8)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7 (29)</td>
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<tr>
<td>Nonimmunized patients</td>
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<tr>
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<td>C1, C2</td>
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<td>Total</td>
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<td>11</td>
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<sup>a</sup> Reactions were considered positive when the 48-hr induration (the mean of 2 diameters) was more than 5 mm.

<sup>b</sup> Numbers in parentheses, total percentage of patients expressing either pre- or postimmunization reactivity.

**DISCUSSION**

We have demonstrated that a course of active specific immunotherapy with an autologous tumor cell-BCG vaccine significantly increased the DCH reactions of patients to autologous tumor cells. Reactions to autologous normal colon mucosa used as a normal tissue control did not increase significantly.

DCH testing is commonly used in the evaluation of cancer patients because the relationship of skin test reactivity to tumor burden, response to therapy, and overall prognosis has been well documented in several malignant diseases (for reviews of DCH testing in humans, see Refs. 4, 18, and 25). In vitro tests of immune function generally do not correlate well with a patient’s clinical condition. Leventhal et al. (17) found a striking lack of correlation in the results of 3 different assays: 51Cr release cytotoxicity; DCH; and lymphocyte stimulation. Of these 3 tests, only the results of skin tests correlated with the clinical state of the patients; the 2 in vitro assays did not correlate with each other. In addition, DCH testing is a simpler procedure than in vitro testing. Tissue culture techniques are not required, and...
cells for testing can be prepared and frozen for future use for serial determinations. The testing can be done in the outpatient setting and causes only minimal discomfort to the patient. However, the generally small DCH reaction makes quantitation less than precise. Bacterial contamination also can be a significant problem for studies using tumors from the gastrointestinal tract, particularly the colon.

Whereas most reports of DCH testing to tumor in humans have used tumor cell extracts or purified antigen preparations, we chose to use whole, cryopreserved, irradiated cells. All testing was done with autologous cells rather than the usual allogeneic cells or cell line. We reasoned that our chances of detecting a response to tumor-associated antigens would be improved by the use of autologous whole cells with any and all putative antigens left reasonably unaltered. Oren and Herberman (20) showed previously that antigenicity was not a function of the way in which the membranes were prepared; intact cells and membranes isolated by other methods trigger similar DCH responses. Baker et al. (1) found a significantly greater DCH reaction to whole autologous, irradiated blast cells than to membrane extracts of those same cells in patients with acute myeloblastic and lymphoblastic leukemia.

Most reported studies of DCH to tumor cells have used only sterile preparations for testing. We accepted the fact that routine bacterial sterility would be impossible with whole, colon tumor cells but reasoned that parallel testing of colon mucosa, which is comparably contaminated, could control for that factor. Although our preparations were contaminated with bacteria, we believe that we have sufficient control data to allow reliable interpretation. The tumor cell and mucosal cell preparations were nearly always contaminated with the same organisms. Yet, there was a significant increase in reactivity between the preimmunization and postimmunization DCH response to tumor cells but not to mucosa cells. If the presence of bacterial antigen was a factor in DCH response, one would expect similar increases in DCH responses to tumor cells and to mucosa cells. Furthermore, 2 patients who were immunized and DCH tested with tumor cell suspensions with no detectable contamination reacted positively to tumor cells. Admittedly, negative bacterial cultures do not assure the absence of bacterial antigens; however, it is the best criterion available for determining whether cell preparations are contaminated. As shown in Table 3, several patients with bacterially contaminated tumor and mucosal cell preparations had negative DCH responses. Thus, the data strongly suggest that the DCH reactions in these patients are the result of sensitization to tumor-associated antigens rather than sensitization to bacterial antigens. Related studies by Stewart (23) support this conclusion. Stewart studied the question of bacterial contamination of cellular extracts used for DCH testing in great detail by adding bacterial antigens to tumor cell preparations. He concluded that the contaminating bacteria were not the cause of the DCH responses induced by the tumor extract in his patients.

There is a possibility that the DCH response to the tumor was simply a sensitization of the body to the tissue of origin rather than a specific reaction to tumor cells. Patients with Hashimoto's thyroiditis have been shown to react to i.d. injections of autologous thyroid extracts (3). Two of our 24 test patients had positive DCH responses to autologous colon mucosa cells 3 weeks after surgery. Seven of 24 (29%) patients reacted to normal mucosa cells after immuno therapy (Chart 2). However, only the increase in DCH reactivity to tumor cells was statistically significant in the overall analysis of our data. The small percentage of patients who demonstrated an increase in their DCH response to mucosa cells may have been sensitized to "tissue-specific" antigens shared by the tumor and mucosal cells. Most of the tumor vaccines were probably contaminated by small numbers of normal mucosal cells that adhered to the tumor at the time of dissociation; thus, one might reasonably expect some increased sensitization to mucosa cells. Another less probable explanation is that premalignant changes in the colon mucosa adjacent to a malignant tumor produce cross-reactivity with the malignant tissue. The importance of having control tissues as close in type to the tumor as possible in order to distinguish between tumor-associated and tissue-associated antigens was demonstrated in the studies by Fass et al. (5) on Ugandan patients with malignant melanoma. These investigators originally reported positive reactions to autologous tumor extracts in 3 patients with localized disease. The detected antigens were believed to be tumor-associated because extracts of autologous lymphocytes produced no reactions. However, in a later study, 6 of 9 patients with positive reactions to tumor extracts also reacted to control extracts from autologous normal skin (2). At least some of the observed reactivity was against tissue-associated antigens present on normal skin and absent on lymphocytes. A study of American melanoma patients showed similar results with membrane extracts and soluble fractions (12). These same investigators demonstrated tumor-associated antigens of colon cancer by skin testing (13). The soluble fraction, obtained by low-frequency sonication of membrane extracts of autologous and allogeneic colon cancer cells and fetal colon cells, produced positive reactions in patients with colon cancer. Comparable preparations from normal colon tissues produced no reactions. Separation on Sephadex G-200 with further fractionation by polyacrylamide gel electrophoresis revealed the skin-reactive antigen in colon cancer to have carcinoembryonic antigen that is different from the carcinoembryonic antigen described by Gold. The qualitative and quantitative differences between the DCH response to tumor cells and to mucosa cells demonstrated in our study suggest that the DCH responses we observed were to tumor-associated antigen(s) with tissue-associated antigen(s) playing a less important role.

The protein concentration of the inoculum is an important variable in DCH testing. Most patients and 76% of the normal volunteers studied by Herberman and Oren (10, 20) had a positive DCH response to autologous leukocyte membrane antigen preparations with high protein concentrations. However, only 10% of the normal volunteers and no patients with tumor had a positive reaction to autologous leukocyte extracts with protein concentrations of less than or equal to 0.33 mg/0.1 ml; none of the responses was positive at 0.1 mg/0.1 ml. Most investigators recommend protein concentrations in DCH test material of no greater than 0.35 mg/0.1 ml to ensure that nonspecific reactions do not occur. However, several investigators (5, 24) have reported that there is no correlation between the protein concentration and DCH reactivity. Our results tend to substantiate the latter conclusion, although most of our DCH test material was below 0.4 mg/0.1 ml (mean of 0.38 mg/0.1 ml for tumor cells and 0.271 mg/ml for mucosa). One patient (Table 3, Patient 1) demonstrated this lack of correlation in that protein concentrations of the tumor cell preparation (0.52 mg) and the mucosa cells (0.116 mg) were very different, yet this was the only patient who reacted to mucosa cells but did not react to...
tumor cells in the postimmunotherapy skin testing. In this patient, tumor cell and mucosa cell preparations were contaminated with *Bacteroides fragilis*. In our study, the mucosa cell preparations had a slightly lower protein concentration than did the tumor cell preparations overall, but the difference is not statistically significant. The fact that patients reacted poorly to skin testing before immunization but had significantly increased reactions both qualitatively and quantitatively after immunization to the same tumor cell DCH preparation indicates that protein concentration was not a key factor in reactivity.

Finally, the role of the enzymes used to dissociate the tumors (collagenase and DNase) should be considered as important variables in the DCH test, since both enzymes are potentially immunogenic. Trace amounts of these enzymes could very well have remained on the surface of tumor or normal mucosal cells. The lower degree of reactivity to normal mucosal cells is a primary reason to exclude the role of the enzymes; however, one of the immunized patients was DCH tested to mechanically dissociated autologous tumor cells (enzyme free), and no significant difference in DCH was detected in comparison to the enzyme-dissociated or mechanically dissociated DCH test cells (48-hr induration of 11 x 8 mm and 16 x 14 mm, respectively). Also, another of the treated patients was DCH tested to autologous tumor cells, normal mucosal cells, an equal volume of supernatant from the cell populations, and an equal volume and concentration of collagenase and DNase. In this patient, a significant DCH reaction was detected only against the autologous tumor cells.

Our goal obviously is to increase the disease-free interval and survival in these patients. Our early results (35 months) with regard to disease-free interval and survival in the treated versus control group are quite encouraging. The following conclusions can be made: (a) Adequate viable tumor cells for an autologous vaccine can be obtained from nearly all Dukes’ Stages B to D colorectal cancer patients. (b) The procedures for vaccine preparation can be conducted in the clinic by trained personnel, such as nurses and technicians. (c) Toxicity is minimal. (d) Acceptance by patients is excellent. (e) Increased immunoreactivity to autologous tumor cells is evident in treated patients. (f) No adverse effects of autologous tumor cell immunization have been found; specifically, there is no evidence of immune-mediated tumor enhancement.

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